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# GROWTH

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# OUTLINE OF A MATHEMATICAL BIOPHYSICS OF CELLULAR FORMS AND MOVEMENTS

*By*

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(*Received June 10, 1938*)

In our previous publications (for a summary see Rashevsky 1936), and in our recent book (Rashevsky, 1938), we have developed, amongst other things, a physico-mathematical theory of various properties and of the behaviour of cells. These developments were based on the theory of mechanical forces, produced in diffusion fields. Therefore on one hand a mathematical study of these forces was required (Rashevsky, 1938, Chapt. VII; Young, 1938), on the other hand a mathematical investigation of the possible effects of these forces was made (Rashevsky, 1936, 1938). To facilitate the mathematical handling of that second category of problems, a number of simplifying assumptions were made. One of the most important simplifications was to confine all considerations only to spherical cells. Inasmuch as a rather large class of cells are at least approximately spherical, the conclusions thus obtained, could be expected to hold at least approximately for some actual cells. As has been shown (Rashevsky, 1936, 1938), we may thus give a qualitative and quantitative account of a number of important phenomena, such as cell-respiration, cell-division, cell sizes and their dependence on various parameters, like rate of metabolism, temperature, etc., the relation between abnormal ratio of cell-division and abnormal glycolytic coefficient, and a number of other phenomena. However the treatment of some of the above problems was in some ways obviously handicapped by the restriction to spherical shapes. Thus, for instance in discussing the theory of cell division, all we could say, is that above a certain size a cell would become unstable and may divide spontaneously in two. But nothing could be said about the process of division itself, during which the conditions of spherical symmetry are not even remotely satisfied.

The restriction to spherical shapes was due to the circumstance that a sphere is about the only shape, for which the differential equation involved can be solved exactly. But if we remember that actual cells anyway are only approximately spherical, one may wonder, whether any *exact* solution of the mathematical equation involved has any biological meaning. The conditions in a living cell are anyway in many respects different from those assumed in setting up our equations, and therefore even if a cell were actually perfectly spherical, our mathematical results would apply to it only approximately. This leads to the idea of attacking our problems in a different way. Instead of solving the mathematical equations *exactly* for such simple and restricted shapes, which only *approximately* resemble real cells, we may try to solve *approximately* the mathematical equations for systems, which are less restricted and do *closely resemble* actual cells. Such a procedure has been recently developed by the author, and

described in the Appendix to his book (Rashevsky, 1938). We shall refer to it hereafter simply as *Appendix*. Not only does this method of approach verify the results previously obtained, but it does bring within its scope a large number of problems, which proved to be refractory to the previous method. The actual process of elongation during division can now be described by a relatively simple differential equation (Rashevsky, 1938, *Appendix*). The principal idea of the new method is the following: Since even two cells, which belong to the same species of cells are only roughly similar to each other, but never are exactly alike, therefore it would not have much sense in describing their shapes even approximately by means of, let us say, the analytical equation of their surfaces. Such a description could anyway never be checked against any observation. What we must pay attention to, are the rough features of the cell, which at once catch the eye of the experimental biologist. Thus if a cell is simply oblong, we may describe it by the ratio of its length 2a to its width 2b (Fig. 1a). If it is approximately egg- or pear-shaped, like for instance *Euglena*, we may describe it by its length $2a$, its maximum width $2b'$ and the approximate angle $2\theta$, that is formed by the "sides" (Fig. 1b). If a cell is "dumb-bell"-shaped, we may approximately describe it by its length $2a$, its maximum width $2b$, and its minimum width $2b'$ at the "neck" (Fig. 1c). The problem then arises to calculate approximately the concentration gradients of the metabolized substances, their diffusion flow and the forces produced by it for such systems, which are characterized only by such rough data as mentioned above. The method of doing this has been developed in the *Appendix* and actual calculations given for some cases.

The purpose of present paper is to *outline* the application of the new method to two important biological problems, which appeared hitherto as being quite hopeless from the mathematical point of view. The first is the theory on non-spherically shaped cells, the second—the theory of internal movements in cells, such as protoplasmic streamings, which sometime result in external movements (Jennings, 1906, p. 3).

## I

That a cell, which contains some rigid skeleton, formed of excretions, may possess a non-spherical form, is easily understood (Thompson, 1917). But there are cells, like *Euglena*, whose interior is undoubtedly liquid, who have no skeletons and yet have a non-spherical shape. Any inert liquid system, imbedded in another liquid medium and subject to no external forces, assumes a spherical shape, due to the action of the surface tension. But if the system is a seat of physico-chemical reactions, as a result of which various diffusion forces appear, then its shape of equilibrium need not be necessarily a sphere (Rashevsky, 1936; 1938, Chapt. X). But what those non-spherical shapes may look like exactly, is a problem practically beyond the reach of present day mathematical methods. Yet, as has been remarked in the *Appendix*, (p. 312) the same method may give us some information on non-spherical shapes, when those are only crudely defined in the way discussed above. In the following developments we shall use the notations of the *Appendix*.

We shall first consider shapes of the type represented on Fig. 1a and consider again the case of a produced substance flowing out. As follows from the discussion in the *Appendix*, in order that such a system should be in equilibrium and neither contract nor elongate, all the three principal stresses $\sigma_1$, $\sigma_2$ and $\sigma_3$ should be zero

$$\sigma_1 = \sigma_2 = \sigma_3 = 0 \tag{1}$$

If we confine ourselves to a system, the permeability of whose surface to the flowing-out substance is very large, then introducing (14) of the Appendix into (35) and (36) and making use of (23), we find:

$$\sigma_1 = \frac{4}{3} A \frac{a^2b^2}{2a^2 + b^2} - \frac{2\gamma}{b}; \qquad \sigma_2 = \sigma_3 = A \frac{a^2b^2}{2a^2 + b^2} - \frac{a + b}{ab}\gamma \tag{2}$$

If we consider however the case of a finite permeability $h$, then we must add to $\sigma_1$, respectively to $\sigma_2 = \sigma_3$, terms resulting from the osmotic pressure at the membrane (Rashevsky, 1938, Chaps. VII and VIII), due to the differences $c_1 - c_0$ and $c_2 - c_0$ (Appendix, p. 317). Moreover, the expression for the longitudinal and transverse diffusion forces per unit volume $f_l$ and $f_t$, are now given not by equations (12) and (13) of the *Appendix* but because of (48) of the *Appendix*, by

$$f_l = \frac{3}{2} \frac{RT\mu}{M} \frac{\bar{c} - c_1}{a}; \qquad f_t = \frac{3}{2} \frac{RT\mu}{M} \frac{\bar{c} - c_2}{b}. \tag{3}$$

Their contribution $\sigma_1'$, $\sigma_2'$ to $\sigma_1$ and $\sigma_2 = \sigma_3$ are, according to (17) and (20), of the Appendix:

$$\sigma_1' = \frac{2}{3} a f_l; \qquad \sigma_2' = \frac{b}{2} f_t \tag{4}$$

Equation (50) of Appendix gives:

$$\bar{c} - c_1 = \frac{ah}{D}(c_1 - c_0); \qquad \bar{c} - c_2 = \frac{bh}{D}(c_2 - c_0) \tag{5}$$

Combining (5) and (3) we obtain:

$$f_l = \frac{3}{2} \frac{RT\mu}{M} \frac{h}{D}(c_1 - c_0); \qquad f_t = \frac{3}{2} \frac{RT\mu}{M} \frac{h}{D}(c_2 - c_0). \tag{6}$$

From (52) of Appendix, we have

$$c_1 - c_0 = \frac{D(\bar{c} - c_0)}{ah + D}; \qquad c_2 - c_0 = \frac{D(\bar{c} - c_0)}{bh + D} \tag{7}$$

From (53), of Appendix, and from (7) we have:

$$(c_1 - c_0) = \frac{2}{3} q \frac{(bh + D)ab}{2ah(ah + D) + bh(bh + D)}$$
$$c_2 - c_0 = \frac{2}{3} q \frac{(ah + D)ab}{2ah(ah + D) + bh(hb + D)} . \tag{8}$$

Introducing (8) into (6), we have:

$$f_l = \frac{RT\mu qh}{MD} \frac{(bh + D)ab}{2ah(ah + D) + bh(bh + D)}$$
$$f_t = \frac{RT\mu qh}{MD} \frac{(ah + D)ab}{2ah(ah + D) + bh(bh + D)} . \tag{9}$$

Equations (9) and (4) together with (23) of the Appendix, give:

$$\sigma_1' = \frac{4}{3} Ah \frac{(bh + D)a^2b}{2ah(ah + D) + bh(bh + D)}$$
$$\sigma_2' = Ah \frac{(ah + D)ab^2}{2ah(ah + D) + bh(bh + D)} . \tag{10}$$

The component $\sigma_1''$ of $\sigma_1$, due to the osmotic pressure at the "ends" (see Appendix, Fig. 1) is equal to

$$\sigma_1'' = \frac{RT}{M} (c_1 - c_0) \tag{11}$$

this being the longitudinal force per unit area, due to $c_1 - c_0$. The component

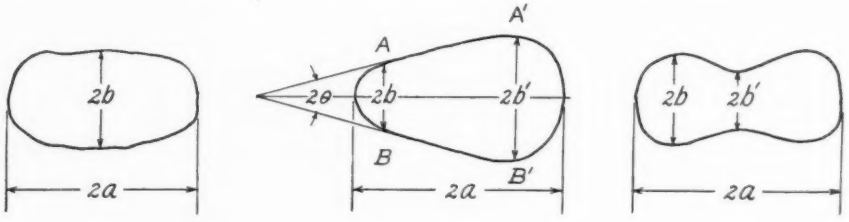


FIG. 1

$\sigma_2''$ of $\sigma_2 = \sigma_3$, due to $c_2 - c_0$, is calculated in a similar way like $\sigma_2$ in (19) and (20) of the Appendix, and is equal to

$$\sigma_2'' = \frac{RT}{M} (c_2 - c_0) . \tag{12}$$

Introducing (8) into (11) and (12) gives:

$$\sigma_2'' = \frac{2}{3}\frac{RTq}{M}\frac{(bh+D)ab}{2ah(ah+D)+bh(bh+D)}\cdot$$

(13)

$$\sigma_2'' = \frac{2}{3}\frac{RTq}{M}\frac{(ah+D)ab}{2ah(ah+D)+bh(bh+D)}$$

Finally the component $\sigma_1'''$ and $\sigma_2'''$ of $\sigma_1$ and $\sigma_2$, due to surface tension, are equal to

$$\sigma_1''' = -\frac{2\gamma}{b}\,; \qquad \sigma_2''' = -\frac{a+b}{ab}\gamma \tag{14}$$

the same as the last terms in (2).

Since $\sigma_1=\sigma_1'+\sigma_1''+\sigma_1'''$ and $\sigma_2=\sigma_2'+\sigma_2''+\sigma_2'''$, the requirement (1) now gives:

$$2A\frac{h(bh+D)a^2b+\frac{D}{\mu}(bh+D)ab}{2ah(ah+D)+bh(bh+D)} = \frac{3\gamma}{b} \tag{15}$$

and

$$A\frac{h(ah+D)ab^2+\frac{4D}{3\mu}(ah+D)ab}{2ah(ah+D)+bh(bh+D)} = \frac{a+b}{ab}\gamma \tag{16}$$

with

$$A = \frac{RT\mu q}{2MD} \tag{17}$$

like in the Appendix.

Dividing (15) by (16) we obtain:

$$6\frac{bh+D}{ah+D}\frac{\mu ha^2b+Dab}{3\mu hab^2+4Dab} = \frac{3a}{a+b}\,. \tag{18}$$

For a given $a$, (18) expresses $b$ in terms of $a$, $h$, $D$ and $\mu$. It does not however contain $A$, which in its turn depends on $q$ and $T$. Hence the ratio $b/a$ of width to length of such an oblong system should not depend neither on the rate of metabolism, nor on temperature. It is also independent of the surface tension $\gamma$. Since for $q=0$, all diffusion forces vanish and under the influence of the surface tension alone, the system must round up (Rashevsky, 1938, Chapt. X), the above result seems somewhat paradoxical. The paradox is however explained by the following considerations. If, by solving (18), we obtain $b$ as a function of $a$, $h$, $D$ and $\mu$ ($b=f(a, h, D, \mu)$) and substitute it then into either (15) or (16), we

shall obtain $a$ as a function of $h$, $D$, $\mu$, $\gamma$, $R$ and $q$. Thus $q$, while not affecting the ratio $b/a$, fixes definitely the size of the cell (for instance the volume $V=(4/3)\pi ab^2$). If $q$ is prescribed, this prescribes $a$ and $b$, and hence also $V$. All the relations derived, in particular (18), which fixes $b=f(a, h, D, \mu)$, will hold only for this particular volume V. Any variation of $q$ will make, *for this particular* $V$, all the above equations incompatible. That is, (1) will not be satisfied and the system will either round up or divide, depending on the variation of $q$.

If in (18) we make $h=\infty$, we find $2=3a/(a+b)$ or

$$b = \tfrac{1}{2}a \tag{19}$$

that is for very large permeability, the ratio $b/a$ is independent even on $D$ and equal to $\frac{1}{2}$. It may be noticed that oblong cells with approximately that ratio of width to the length are quite usual. If in (18) we make $h=0$, we find:

$$b = a \tag{20}$$

that is the system rounds up. If we introduce (19) into either (15) or (16), we find after simple calculations:

$$a = \sqrt{\frac{54MD\gamma}{RT\mu q}} = 1\cdot 82a^* \tag{21}$$

where $a^*$ is given by (34) of the Appendix. Equation (21) shows, that when $q$ becomes zero, $a$ becomes infinite. So that while the ratio $b/$ does not depend on $q$, yet for $q=0$ no finite oblong cells can exist.

The situation becomes somewhat different, when we consider forms, like those represented on Fig. (1b). Instead of $2a$, $2b'$ and $2\theta$ we may characterize it *approximately* by $2a$, $2b'$ and $2b\sim AB$. The angle $\theta$ is then given approximately by

$$\cos\theta = \frac{2a}{\sqrt{4a^2+(b'-b)^2}}. \tag{22}$$

The average concentrations and the forces in such a system are approximately the same as in a simple oblong system (Fig. 1a), in which instead of $b$ we substitute $\frac{1}{2}(b+b')$. However the longitudinal component of the surface tension force which contributes to $\sigma_1'''$ is obtained by multiplying the previous values by $\cos\theta$, so that now we have instead of (14):

$$\sigma_1''' = -\frac{4\gamma}{b+b'}\cos\theta = -\frac{8a\gamma}{(b+b')\sqrt{4a^2+(b'-b)^2}}. \tag{23}$$

Since we are discussing here this case only as an *illustration* of the method, we shall confine ourselves to the special case of $h=\infty$. The requirement (1) then gives:

$$\frac{4}{3}A\frac{a^2(b+b')^2}{8a^2+(b+b')^2} = \frac{8a\gamma}{(b+b')\sqrt{4a^2+(b'-b)^2}} \tag{24}$$

and

$$A\frac{a^2(b+b')^2}{8a^2+(b+b')^2}=\frac{2a+b+b'}{a(b+b')}\gamma. \tag{25}$$

Dividing (24) by (25) we have

$$\frac{8a^2}{(2a+b+b')\sqrt{4a^2+(b'-b)^2}}=\frac{4}{3}. \tag{26}$$

The volume $V$ of the system may be expressed through $a$, $b$ and $b'$. For instance approximately we may use the expression for a truncated cone, giving

$$V=\tfrac{2}{3}\pi a(b^2+b'^2+bb'). \tag{27}$$

From (26) and (27) we may express $b$ and $b'$ as functions of $a$ and $V$. Thus

$$b=f_1(a,V);\qquad b'=f_2(a,V). \tag{28}$$

Introducing this into either (24) or (25), we obtain an equation, which expresses $a$ as function of $V$, $q$, $T$, $h$, $D$, $\mu$ and $\gamma$. Hence for any *given* volume $V$ we shall have $a$, $b$ and $b'$ determined as a function of the rate of reaction $q$ and of the other parameters. Thus for a given volume, the shape of the cell is a continuous function of the rate of reaction. It must however be remarked that (26) and (27) may not have real positive roots for some values of $V$. Approximate calculations show however, that the range of values of $V$ for which solutions exist, correspond to the range of values for the volume of actual cells ($10^{-8}-10^{-9}$ cm.$^3$).

Equations (26) and (27) are not convenient for analytical handling. We shall use here a very rough and rather objectionable approximation, in order to *illustrate only* the type of results one may arrive at in such considerations. An improved and more detailed treatment of these problems will be given in future publications.

If $\theta$ is rather small, then we may neglect $(b'-b)^2$ as compared to $4a^2$ in (26). The former then gives:

$$a=b+b'. \tag{29}$$

Together with (27), (29) gives after simple calculations:

$$b=\frac{a}{2}-\frac{\sqrt{\frac{6Va}{\pi}-3a^4}}{2a};\qquad b'=\frac{a}{2}+\frac{\sqrt{\frac{6Va}{\pi}-3a^4}}{2a} \tag{30}$$

$b$ and $b'$ are real only if $6Va/\pi>3a^4$ or if

$$a^3<\frac{2V}{\pi}. \tag{31}$$

On the other hand obviously $a$ must be larger than $a_0$, the radius of a sphere having the volume $V$. Since $a_0^3 = 3V/4\pi$, we have

$$\frac{3V}{4\pi} < a^3 < \frac{2V}{\pi}. \tag{32}$$

Substitution of (29) into (25) or into (26) with the corresponding neglection in the radical gives for $a$ the expression (21). As $q$ increases, $a$ decreases and therefore, as seen from (30), the difference:

$$b' - b = \sqrt{\frac{6V}{\pi a} - 3a^2} \tag{33}$$

increases. Hence with increasing $q$, the shape of a cell should become more asymmetrical, less "pointed" at one end, the angle $\theta$ increasing. In this way we may study theoretically the shape of cells as a function of other constants, such as $T$, $h$, $D$, $\gamma$ and thus suggest new problems for the experimenter.

## II

We shall briefly outline the way of treating by the proposed approximate method another important biological problem, which proves to be quite refractory to an attack by the usual exact method of differential equations.

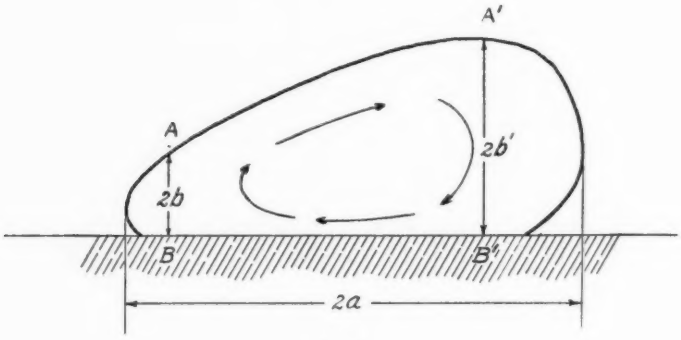


FIG. 2

Let us consider a case discussed previously qualitatively (Rashevsky, 1934, p. 197) and represented on Fig. 2. Here we have a streaming of the cell cytoplasm due to the asymmetrical distribution of diffusion pressures. Mathematically this reduces to a complex problem in differential equations of diffusion with superimposed convection, a problem that still awaits its solution. Following the principle that an approximate solution of a problem is always better than no solution at all, we shall very briefly outline here an attack on that problem, based on our new method.

Consider a case of substance diffusing into the cell. If the cell lies, as considered before (Rashevsky, 1934, p. 197) on a solid surface, the maximum concentration will be at the "top" surface of the cell. But the average concentration $\bar{c}$ in the region $AB$ will be different from the average concentration $\bar{c}'$ in the region $A'B'$. Let us again for simplicity consider the case of $h = \infty$. Consider the vertical component of the transport of the substance at $A'B'$. The average diffusion flow per cm$^2$ is of the order of $D(c_0 - \bar{c}')/2b'$. If $v$ denotes the average velocity of the streaming, then the average convection flow per cm$^2$ is (Rashevsky, 1938, p. 13) of the order of $v\bar{c}'$. The total flow per cm$^2$ is in the stationary state of the order of the total consumption in a column of height $2b'$ and of one cm$^2$ cross-section, that is $2b'q$. Hence approximately

$$D\frac{c_0 - c'}{2b'} + v\bar{c}' = 2b'q. \tag{34}$$

Similarly, at $AB$

$$D\frac{c_0 - \bar{c}}{2b} - v\bar{c} = 2bq. \tag{35}$$

The minus sign before $v\bar{c}$ is due to the fact that at this end of the cell the convection current flows in the opposite direction to the diffusion current. Equations (34) and (35) give

$$\bar{c} = \frac{b^2q - Dc_0}{bv - D}; \qquad \bar{c}' = \frac{b'^2q - Dc_0}{b'v - D}. \tag{36}$$

If now we have an equation, connecting $\bar{c}$ and $\bar{c}'$ with $v$, we shall be able to determine approximately all three quantities. This last equation will depend on the physical assumptions, concerning the forces exerted on the cytoplasm in a diffusion field. Let the total driving force, which produces the protoplasmic streaming be $F(\bar{c}, \bar{c}')$. The resistance to the flow is produced by the viscosity $\eta$. Remembering, that the average difference in velocities of the upward flow at $AB$ and the downward flow at $A'B'$ is approximately $2v$ and the average distance between the two is $a$, we have for the resistance force of shear per cm$^2$ $2v\eta/a$. The total resistance force is obtained by multiplying this approximately by the transverse cross-section area, which is of the order of magnitude of $\pi/4(b+b')^2$. In the stationary state the force of resistance is equal to the driving force. We therefore obtain as third equation

$$\frac{\pi v\eta(b + b')^2}{2a} = F(\bar{c}, \bar{c}'). \tag{37}$$

If, for instance we make $F(\bar{c}, \bar{c}')$ equal to the difference of the diffusion pressures at $AB$ and $A'B'$, which is equal to the difference of pressures per unit area, multiplied by half the longitudinal cross-section, we shall have:

$$F(\bar{c}, \bar{c}') = \frac{RT}{M}(\bar{c} - \bar{c}') \times a\frac{b + b'}{2}. \tag{38}$$

38) and (37) give

$$\pi v\eta(b + b') = \frac{RT}{M} a^2(\bar{c} - \bar{c}'). \tag{39}$$

Together with (36), (39) gives:

$$\pi v\eta(b + b') = \frac{RT}{M} a^2 \left( \frac{b^2 q - Dc_0}{bv - D} - \frac{b'^2 q - Dc_0}{b'v - D} \right) \tag{40}$$

which is an equation determining $v$, the algebraic sign of $v$ determining the direction of streaming.

The assumption (38) is not a very plausible one and is considered here only as an illustration. We may consider gravitational effects, due to the fact that the specific weight of a solution usually increases with the concentration (Rashevsky, 1934, p. 196). This leads to similar equations. The direction of the flow will of course also depend on the assumptions made. We may introduce also temperature effects.

In a similar way we may study other types of cytoplasmic streamings for instance those observed during the cell-division.

The principal result of this paper is that it shows how, by a method of approximation such problems like the form of cells and the protoplasmic streamings, which are exactly described by most complex differential and functional equations can be reduced to algebraic equations.

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# FREE AMINO ACID LOCALIZATION IN *OBELIA GENICULATA*

*By*

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The colonial-living form of the marine hydroid *Obelia geniculata* is used in this laboratory as a test animal for evaluation of the part played in growth by the naturally occurring tissue components of general distribution (1). The results are interpreted on three assumptions, viz.—that the compounds are ingested by the animal; that they are distributed to growing parts; and that dissolution of regressive hydranths is expression of an endogenously determined catabolism (2–4).

The original purpose here was to see if there is chemical evidence of digestive activity in the gastral cavity (5), and of catabolic disintegration in the "senile" animals. As often happens one thing has led to another so that additional data on intermediary and growth metabolism can also be given.

## PROCEDURE

Only freshly collected healthy and uninjured colonies were used. They were treated throughout with the meticulous care outlined in earlier papers (6: 7).

Four reagents were used in tracing amino acid distribution, viz.—ninhydrin for free carboxyl and adjacent $-NH_2$; phosphotungstate-phosphomolybdate mixture for tyrosine; bromine-water for tryptophane; and sodium nitroprusside for $-SH$ (8).

Two series of observations were made: one on the material as collected and one on colonies kept intact on *Mytilus* while exposed to the following amino acids in the stated concentrations: glycine (1/100,000), l-tyrosine (1/100,000), l-tryptophane (1/100,000), glutathione (1/100,000), l-cysteine (1/100,000), l-cystine (1/100,000). These were compared with controls kept simultaneously under like conditions save the presence of added amino acid. Test and control colonies were matched as far as possible with respect to amount and kind of pigmented material; and number and state of development of the hydranths.

For study a suitable colony was placed on a glass slide and while under observation it was brought uniformly in contact with the given reagent—either by mixing with or after previous removal of the sea-water as the case demanded. Color development was followed under the microscope using that illumination which best brought out differential intensity. It was found that interposition of lemon-yellow coated paper on the microscope stage between condenser and slide aided vizualization of blue. Due care was taken that tissue distortions arising from reagent action were adequately discounted in coming to decision as to localization of the reactants.

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## RESULTS

*Free Amino Acids:* Using 0.4% ninhydrin in the cold and fresh cut colonies evidence was given of amino acid localization in the endoderm of the bud tip; in the distal endoderm of the half-stage hydranth; in the endoderm of the tentacle primordia of the three-quarter-stage hydranth; and in the inner cell layer of the tip of the outgrowing stolon. No consistent difference in free amino acid concentration was shown by the several growing regions. Possible but uncertain was occasional concentration in the distal hydroplasm of growing parts. In colonies exposed to glycine for from one to three hours and rinsed twice in seawater the above reactions were definitely heightened and a strong positive test was given in distal hydroplasm. This is evidence that free amino acids are distributed to growing parts by the hydroplasm. Plate I, Figs. 1–4: 11.

Always in the mouth tissue was there a strong blue coloration. The oesophagus—if the pedicular connection between mouth and gastral cavity may be so called—was similarly reactive. In the gastral cavity itself there was often a faint diffuse blue with more intense reaction on the inner tissue surface, especially on the upper curvature. In rinsed glycine-exposed colonies a more intense reaction was given. It is evident that digestion occurs either on the surface of the mouth or in the gastral cavity or both and that amino acids are ingested and concentrated by the mouth and gastral cavity. Plate I, Fig. 5.

The center of the senile structures gave a blue which diminished from within outwards to negligibility at the periphery. Such was even found in the last vestige of hydranth substance left in the hydrotheca. This is evidence that the regressing hydranths undergo senile resolution through an endogenously determined catabolism. No increased reaction was given in seniles on colonies exposed to glycine. Plate I, Figs. 8–9.

After long standing, or on warming, a more or less generalized surface purpling took place. At times this was more intense at and around pedicular junctions with the hydrocaulus. The color difference, the wide distribution, the loci of concentration, and the necessity for time lapse or warmth, suggest this reaction to be derived from something other than free amino acids.

Now amines other than as found in amino acids may give a color with ninhydrin if they are in certain combinations (8); and chitin which invests *Obelia* is composed of glucosamine (9). It is probable that this or some closely allied compound is responsible for the non-amino acid generalized reaction since the compound is necessary for chitin formation during growth and may result from chitin disintegration by enzyme or other agent.

*Tyrosine:* The distal endoderm of developing hydranths gave a positive reaction to the phosphotungstate-phosphomolybdate mixture as it did to ninhydrin. The intensity was definitely and consistently greater in animals at the three-quarter-stage. It was more marked at the site of stolon metaplasia than in the distal portion of the non-metaplasic stolon. Plate I, Fig. 12. A positive reaction was present at the site of long bud emergence. Plate I, Fig. 2. Although the hydrocaulal endoderm was faintly blue in some colonies this was by no means consistent. The concentration was in distal growing endoderm, not in

that of the hydrocaulus. The hydroplasm showed no blue. In colonies exposed to l-tyrosine for from one to three hours and twice rinsed in fresh sea-water the reaction was intensified in the three-quarter developed hydranths. It was occasionally exhibited in the hydroplasm. It was more frequently found in the endoderm of the hydrocaulus and in greater intensity. All this is evidence that tyrosine is distributed by the hydroplasm.

Always was the mouth deeply blue. The oesophagus also. Coloration of gastral contents was not seen; but on occasions the gastral cavity surface took a deep blue. The tips of tentacles in contact with the mouth were intensely blue; but the free-waving members were colorless. In colonies exposed to l-tyrosine and twice rinsed the mouth reaction was intensified and there appeared a diffuse blue coloration in the gastral cavity. All this is evidence that digestion takes place in mouth and gastral cavity of functionable obelias and that tyrosine is ingested and concentrated thereby. Plate I, Figs. 6–7.

Senile hydranths reacted the same here as in the ninhydrin experiments. By good fortune there presented itself within a hydrotheca a disintegrating hydranth which had lost all connection with its pedicular hydroplasm. And here the blue color—chemical evidence of protein dissolution—instead of being within the center of the mass was on the outer surface. This is further evidence that the normal course of hydranth senile resolution is determined by endogenous catabolism (3). Plate I, Fig. 10.

*Tryptophane:* The violet color which is given when tryptophane in weak solution is exposed to bromine is faint and fugacious. Because of this and because tryptophane is hardly a large percentage of the amino acid milieu of obelia it was only possible to detect its presence in the gastral cavity and the tissue mass of animals undergoing senile resolution. In these the test came stronger in the center and faded into nothingness towards the periphery. Plate I, Figs. 5: 8.

In colonies exposed to l-tryptophane for from one to more hours and twice rinsed the reaction within the gastral cavity was greater than control. In addition both the hydroplasm within the hydrocaulus—or possibly the endoderm therof—and the mouth tissue showed positive. No increase occurred within the senile animals.

All this is evidence that digestion takes place within the gastral cavity of obelia hydranths; that tryptophane like other amino acids is ingested and concentrated thereby; and that the dissolution of regressive hydranths is expression of endogenous catabolism.

*Glutathione; Cysteine; Cystine:* The localization of −SH in obelia has been described by the junior author (7). All that need be written here is that free −SH is found concentrated wherever cell proliferation is presumedly taking place; that the intensity of reaction is greater where cell increase in number is assumedly greater, and less where it is less; that mouth and gastral cavity give evidence of digestive production of SH-containing protein split-products; and that similar substances are present in hydranths undergoing senile resolution. These observations have been repeated and confirmed. There only remains

the report of exposure to naturally occurring sulfur-containing amino acids.

The colonies used for the glutathione experiments were not particularly good feeders. This was shown by the fact that the tentacles of most of the complete hydranths were withdrawn into the hydrothecae for long periods. As a result growth activity was less and but faint traces of −SH reaction obtained in the controls and these were confined to the buds and gastral cavities. In 1/50,000 and 1/100,000 glutathione, however, the reaction was enhanced in these places and extended to the distal endoderm and hydroplasm of half and three-quarter developed animals. This happened despite the fact the animals did not seem to feed as readily in glutathione as in control solutions.

No more than the control increase in reaction intensity was produced when colonies exposed to glutathione were put in 1 percent KCN for 10 minutes prior to application of the nitroprusside test. Hence no evidence of R-S·S-R formation from G-SH was obtained.

In the cysteine (not cysteine hydrochloride) set both test and control colonies were good feeders. Within half an hour or more colonies exposed to 1/50,000 and 1/100,000 concentration showed free −SH in amount higher than control in mouth and gastral cavity; hydroplasm and hydrocaulal endoderm; endoderm of buds, half-stage and three-quarter developed hydranths. Bud reaction was earlier and in higher concentration than in the later developmental stages.

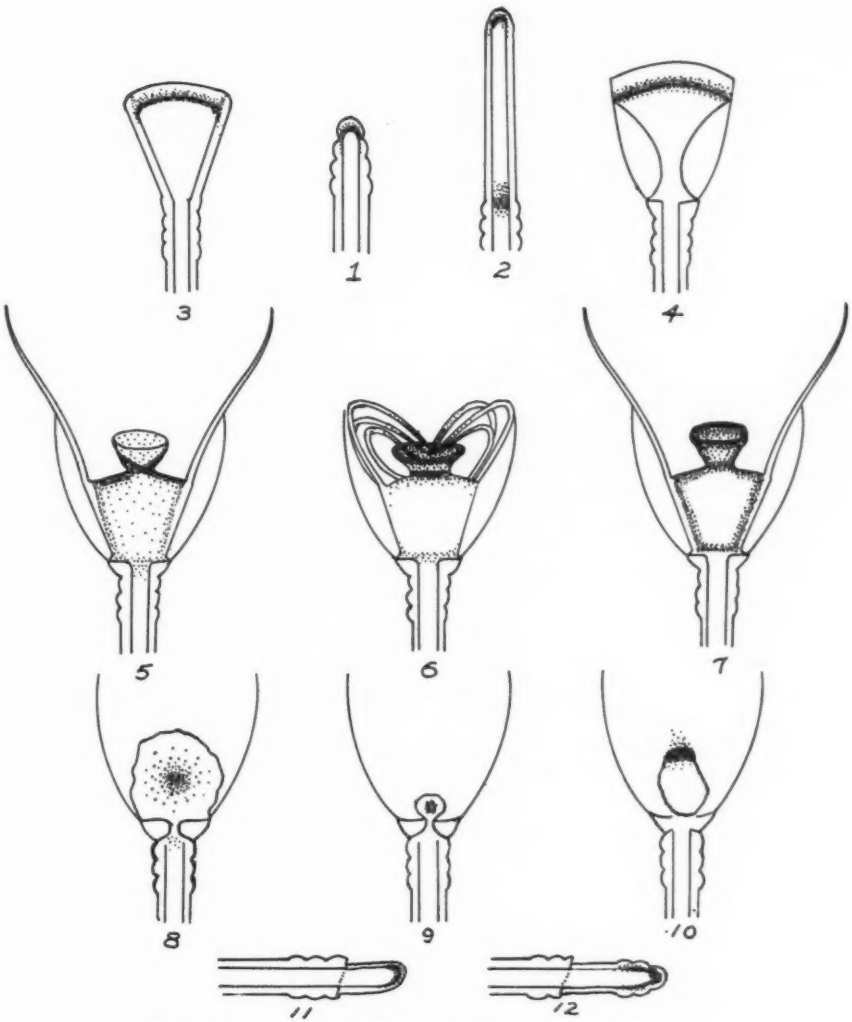
With respect to cystine two questions are presented. Is the compound ingested and distributed as are other amino acids? Does the ingested disulfide remain as such or is it reduced to sulfhydryl?

It is convenient to first give the result of the last query.

When intact obelia colonies on *Mytilus* are taken from 1/50,000 and 1/100,000 cystine in sea-water after from one to three hours and twice rinsed in sea-water, they show greater concentration of free −SH in the usual localizations—seniles excepted—than do control colonies not exposed to cystine. Noteworthy is the concentration in distal hydroplasm of growing structures and the fact repeatedly confirmed that test buds and half developed animals are much more roseate than control.

Careful testing of the cystine containing solutions gave no evidence of free −SH therein. Evidently cystine is ingested by obelia and the disulfide group reduced to sulfhydryl.

When cystine exposed colonies are tested for disulfide by nitroprusside after 10 minute reduction with 1 percent KCN a different picture evolves. Consistent test superiority to control occurred only in mouth and gastral cavity. Occasionally a flash of pink in the general hydroplasm was elicited which was not found in control colonies. But that is all. Indeed color development in the growing parts was as often less in the test colonies as it was greater than control. This result in the face of the known superiority of test colonies in free −SH, as demonstrated by nitroprusside without preliminary cyanide reduction is evidence that −SH was destroyed probably by the excess alkalinity (10). The finding suggests that cystine as such is taken in by mouth and gastral cavity but does not get beyond to any marked extent.




These figures show the localization of free amino acids in Obelia as given by the chemical tests

FIG. 1. Amino acids in bud endoderm.
FIG. 2. Tyrosine accumulation at site of long bud emergence.
FIGS. 3–4. Amino acid concentration in endoderm of half and three-quarter developed animals
FIG. 5. Amino acid localization in mouth and gastral cavity of mature animal.
FIG. 6–7. Tyrosine concentration on tentacle tips and in mouth and gastral cavity of mature animals.
FIGS. 8–9. Amino acid concentration within animals undergoing senile dissolution with pedicular-hydroplasmic connection intact.
FIG. 10. Disintegration from without of senile hydranth unconnected with the colony.
FIG. 11. Amino acid concentration of stolon endoderm.
FIG. 12. Tyrosine accumulation at site of stolon metaplasia.

It is clear from these results that glutathione, cysteine, and cystine are ingested and distributed by and in obelia as are other amino acids.

*Summary of Results:* The data here presented show that amino acids are ingested by obelia hydranths; that they are concentrated thereby from their dilute sea-water solutions; that they are distributed by the hydroplasm and concentrated in the growing parts; and that they are liberated in the disintegration of "senile" hydranths.

## DISCUSSION

For coherence and clarity attention is drawn here rather than under procedure to certain technical matters important to interpretation.

During these winter investigations it has been observed that the feeding activity of obelia communities exhibits diurnal variations even under laboratory conditions. This is undoubtedly a tidal rhythm association (11). The material was collected at low tide, usually early in the morning. The animals fed up to about ten o'clock when they entered into a rest period with tentacles withdrawn into the hydrothecae. Along about four o'clock in the afternoon tentacles were again put out and feeding resumed. Attempts to prolong feeding time by changes in temperature or illumination were futile. Change into fresh sea-water sometimes gave a transitory stimulation to feeding; but this was uncertain.

Now observations made during and shortly after feeding were consistent and clean-cut, whereas attempts at amino acid localization beginning from three to four hours after feeding had stopped frequently gave variable results. These occurrences set a procedure and gave information of growth metabolism. They showed that feeding or just-fed animals must be used if complete information is to be had of amino acid ingestion, distribution, and localization. And they showed that feeding, digestion, and utilization is not continuous but intermittent with periods of supply alternating with periods of depletion. This is demonstration that the mechanisms of obelia nutrition are the same in principle as those of the so-called higher animals.

The chief channel of amino acid entry into obelia is by oral ingestion, not by absorption through stolon, hydrocaulus, or undeveloped hydranths.

The indirect evidence is that mechanism for ingestion is had in tentacles, mouth, and gastral cavity; that activity of ingestion is observable in movements of tentacles and mouth; that solid products of ingestion can be seen in the gastral cavity.

The direct evidence is chemical demonstration of free amino acids in mouth and gastral cavity; the increased amount in gastral cavity when feeding animals are exposed to amino acid containing culture solutions; the absence of such gastral increase in animals which have not fed; the decrease in reaction intensity with time throughout non-feeding animals exposed to amino acids; the relatively high concentration of amino acids within the animal in contrast to the dilute culture solution of much weaker reaction intensity; and the absence of amino acids in the extra-vascular fluids of animals kept in amino acid cultures.

Amino acids are not only ingested by obelia, they are concentrated thereby from their dilute solution in sea-water.

The evidence is the truly great reaction intensity in mouth tissue of animals exposed to amino acids; the occurrence of a greater reaction intensity in gastral content and hydroplasm than in the culture solution from which they are derived; and the differential reaction intensity of endoderm in contrast to other tissues.

Ingested protein material is digested and broken down into amino acids by obelia as by higher animal forms. Solid particles are frequently seen in the gastral cavity and adhering to the mouth. These are bits of amorphous material and disintegrating small organisms. When the several tests are applied an outer zone of these particles often takes a good color. This is evidence of proteolysis. Since it occurs around rather than in the particle it indicates digestion is effected by obelia produced proteolytic enzymes rather than by those introduced by the ingesta.

The source of these enzymes is not established. But since free amino acids are chemically demonstrable in mouth tissue and contents to greater consistency and clarity than in any other part of the alimentary system; and since those distal parts of tentacles which bending inwards are in actual physical contact with the mouth and those only give a strong positive reaction with certain reagents, the tentative assumption seems justified that what is called mouth tissue is a significant contributor to proteolytic enzyme production.

Along with this goes the observation that occasionally particles may be seen bobbing around in the pedicular hydroplasm contiguous to feeding hydranths which also yield an outer zone of free amino acid reaction.

This and the presence of similar particles in the gastral cavity, taken together with the localization of free amino acids in mouth tissue and the increased concentration therein in animals exposed to amino acid containing cultures, extend the suggestion of oral proteolytic production considerably. For it suggests that ingested material becomes invested with proteolytic enzymes by first coming in contact with the oral entoderm. That some part of this material is dissolved *in situ*. But that owing to contractile and other as yet unrecognized activities particles are carried into the gastral cavity where digestion is continued with or without additional supply of enzyme from gastral tissue. And that from this some particles the digestion of which has not been completed but which still carry their investment of proteolytic enzyme may escape into the pedicular hydroplasm.

The hydroplasm is the means whereby the amino acids resulting from protein digestion and those ingested and concentrated from culture solutions are distributed throughout the colony.

Mechanical evidence is the hydroplasmic streaming away from feeding and senile hydranths and towards and into growing ones (3). Chemical evidence is the occasional reaction for free amino acids given by the hydroplasm of colonies exposed to amino acid containing cultures, and the frequent reaction of the hydroplasm just below the gastral cavity thereof; the occasional reaction of

hydroplasm in developing animals and of particles bobbing around therein; and the not infrequent concentration of amino acids in the hydroplasm contiguous to the distal endoderm of growing parts which increases in colonies kept in amino acid containing cultures. Other evidence is the occasional reaction of hydrocaulal endoderm in such colonies. It is inconceivable that this tissue could have gotten its free amino acids from any other source than the hydroplasm since no test was ever given in the extra-vascular fluids or spaces.

The occasional presence in the hydroplasm of developing animals of particles giving a surface test for free amino acids raises a nice question. Is this derived from proteolysis of the reacting particle or is it derived from amino acids adsorbed thereon from the hydroplasm?

We have obtained no impression that either the number or intensity of reaction of these particles is greater in positive reacting hydroplasm of colonies kept in amino acid culture solutions than in negative reacting hydroplasm of controls. Because of this—even though discrimination is uncertain—we incline to the tentative belief that the reacting particles express prolonged proteolysis rather than adsorption, though the latter possibility is not ruled out.

Whether or not this means the hydroplasm is part of the alimentary system is a matter of interpretation. It is of course not impossible that there are cells in the hydrocaulal endoderm which may be facultatively productive of proteolytic enzymes for digestion of ingested material which has escaped dissolution in mouth and gastral cavity. But even if this be so it is quite evident that digestion is more largely a property of mouth and gastral cavity than of hydrocaulal tissue, while a chief function of hydroplasm is distribution.

The distributed amino acids are specifically utilized in growth.

The evidence is the positive correlation between growth activity and free amino acid localization; the positive correlation between growth activity and free amino acid concentration; the increase in free amino acids in developing tissue and hydroplasm contiguous thereto when feeding colonies are kept in amino acid containing culture solutions, and the absence of increase in colonies which do not feed; and finally the decrease in free amino acids in developing hydranths and distal hydroplasm of test and control colonies towards the end of the non-feeding rest period.

The gradual shrinkage to disappearance of hydranths which have ceased function and withdrawn into the hydrothecae is due to endogenous catabolic dissolution.

Mechanical evidence is the downstreaming of the pedicular hydroplasm away from the disintegrating hydranth (3). Chemical evidence is the presence of amino acids within rather than at the periphery of the mass; the extension of reaction down into the hydroplasm immediately adjacent thereto rather than in any other direction; and the fact that the reaction was not increased *within* senile animals of colonies kept in amino acid containing culture solutions.

This is the picture when there is physiological connection between dissolving hydranth and the rest of the colony through pedicel and hydroplasm. A different picture comes when there is no such connection. Here the pedicular hydro-

plasm may be distally directed—particularly when a new bud is being formed on the site of the old animal by recurrence—and it is the outer surface rather than the inner core which gives the amino acid reaction. There is little if any evidence of inner proteolysis in the disconnected mass lying discrete within the hydrotheca. The contrast between the localization of dissolution in the two cases is evidence consistent with the postulate that senile hydranths are normally removed by endogenous catabolism. For where there is no physiological connection dissolution is taking place from without inwards and where such exists dissolution is from within outwards.

In view of the fact that the disintegration products are carried away to the rest of the colony by the downstreaming hydroplasm; the fact that these are identifiable as amino acids; and the fact that amino acids are found concentrated at points of active growth; the tentative conclusion is advanced that the colony uses the products of dissolution of its senile members for the building of new animals.

Correlation of the structural, physiological, and *in vivo* chemical evidence presented in this and preceding papers establishes beyond question that obelia follows if it does not set the classic procedure with respect to ingestion, preparation, distribution, concentration, and utilization of the amino acid building material for growth. Protein is taken in and broken down into its constituent amino acids in a digestive system; it is distributed throughout the colony as amino acids in a vascular system; it is concentrated as amino acids in tissue where growth is active; and these are incorporated therein as new protein is built into the new structures. It is clear that the animal is good new material for many inquiries into the chemistry of growth and nutrition.

Coming from the general to the particular, comment is in order on the possible relation of differences in distribution of amino acid concentration intensity to developmental growth.

Tryptophane eliminates itself because its detection by bromine as used here was uncertain save in gastral cavity and senile interior.

The amino acid picture revealed by ninhydrin gave no evidence that any particular stage of obelia development differed from any other with respect to general amino acid concentration.

The story is otherwise with respect to tyrosine and the sulfhydryl carrying amino acid.

Now it is a fact that the phosphotungstate-molybdate reagent for tyrosine may also be reduced by sulfhydryl in certain concentration under certain conditions. And it is obviously impossible to so regulate conditions when making *in vivo* tests such as these with both sulfhydryl and tyrosine present that one can be sure the blue color which follows phosphotungstate-molybdate is due to tyrosine alone or even at all.

Certain repeatable differences in distribution of reaction intensity to the two reagents do however allow conclusion that tyrosine is a reactant in one particular instance. Thus nitroprusside shows highest sulfhydryl concentration in buds and lowest in three-quarter developed animals; while phosphotungstate-molyb-

date shows but weak blue in the buds and a most strong color in the three-quarter grown animals. Further, whereas the nitroprusside reaction is commonly less in the half stage hydranths than in the buds; the phosphotungstate-molybdate reaction is less in the buds and greater in the half developed animals. These increases in reaction intensity to phosphotungstate-molybdate cannot be due to sulfhydryl since this decreases from one developmental stage to the next. It is therefore just to conclude that tyrosine accumulation is demonstrated in the three-quarter developed hydranth.

This is significant.

In the first place the reaction is essentially specific to tyrosine. It is not shown by amino acids in general, nor is it shown by sulfhydryl carrying acids in particular. Further it begins in the larger or late half stage, comes to an apparent maximum in the early three-quarter, and seems to diminish more often than not as this progresses towards organization into functionable hydranths.

At the very beginning of this work it was claimed from the nature of the structural changes that differentiation is the dominant growth activity concerned in the progression from the half to the three-quarter stage (6). And the amino acid growth studies show that l-tyrosine acts definitely to forward the expression of this change (12).

The *in vivo* chemical demonstration of tyrosine concentration at just the time and place where this particular structural change is taking place is therefore decisive evidence that the progression is largely dominated by the growth activity of differentiation, and that tyrosine is a specific participant in some process essential thereto.

Supplementary evidence is the fact that tyrosine was also found concentrated where stolon metaplasia was in progress, a phenomenon which is obviously factored by differentiation.

Just as the postulated change in differentiation activity expression is paralleled by demonstrable change in tryrosine concentration, so is the postulated decrease in proliferation activity from its high point in the bud to its low in the three-quarter developed hydranth paralleled by a concomitant *in vivo* decrease in sulfhydryl.

This is pretty direct evidence not only that the primary assumption as to the relative extent of proliferation activity in the successive growth stages of an obelia hydranth was correct; but also that the sulfhydryl group is a specific participant in some process essential to cell increase in number (13).

This parallelism has reports from other sources, references to which are given elsewhere (7).

There is no question but that a nicer demonstration of sulfhydryl in proliferating parts is obtainable in winter than in summer. When it is remembered that the winter data are obtained from animals transferred from the growth-retarding ice water of the natural habitat to the growth-forwarding warming water of the laboratory, while the summer data are factored by no such temperature differential with its growth-releasing effect, it is clear that one more piece of

evidence is here added to the postulate that proliferative growth activity and sulfhydryl are functionally related.

Also of interest is the fact that a good part of the sulfur of ingested cystine is reduced to sulfhydryl before being passed on to the hydroplasm, and that it is in this form it is distributed to the proliferating parts rather than as disulfide.

In conclusion it may be noted that the correlation between tyrosine and sulfhydryl localized concentration and the structural forms taken by an obelia hydranth during its life cycle from anlagen to empty hydrotheca corroborates the postulate concerning the nature of the developmental activity dominant in each successive morpho- and histogenetic change (14).

## SUMMARY AND CONCLUSIONS

Chemical tests for free amino acids in general and for tyrosine, tryptophane, and the −SH and −S.S− groups in particular have been made under the microscope on living colonies of *obelia geniculata* as taken directly from their natural habitat and after longer or shorter periods of feeding in amino acid containing culture solutions.

Due account was taken of the feeding activity and rhythm, the presence of pigmented material within the colonies, the structural distortions arising from reagent action, and the size and developmental state of the colonies.

Amino acids were normally found localized in mouth, oesophagus, and gastral cavity; in the distal endoderm of growing parts; and in the core of senile hydranths. In colonies fed amino acids the intensity of reaction was enhanced in the stated places—seniles excepted—and reaction was given in the hydroplasm, particularly the distal portions contiguous to the growing parts, and the hydrocaulal endoderm. No reaction was given in the extra-vascular spaces or fluids.

There was positive correlation between tyrosine concentration and foci of differentiation; and there was positive correlation between intensity of −SH reaction and proliferation expression.

The data allow the conclusion that amino acids are not only ingested by obelia; but that they are the natural products of digestive enzymes produced by mouth and gastral cavity; that they are concentrated thereby from the surrounding medium; that they are passed on to the hydroplasm and distributed thereby throughout the colony; and that they are specifically utilized in growth.

The data strengthen the previously expressed belief that tyrosine is a specific participant in some process essential for expression of differentiation; the postulate that sulfhydryl is of signal importance to proliferation; and they give chemical evidence that the senile regression of worn out hydranths is accomplished by endogenous catabolism.

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# THE RELATIVE GROWTH OF PARTS IN THE BLUE CRAB *CALLINECTES SAPIDUS* RATHBUN

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## INTRODUCTION

This paper deals with the extent of individual variations in size of certain dimensions of *Callinectes sapidus* and the quantitative nature of the growth dimensional ratios that characterize this crab. The problem represents an outgrowth of an attempt to ascertain the average number of moults in the life history of the organism, which subject will be treated in a subsequent paper.

Despite the fundamental biological significance of studies on differential growth rates, comparatively little concerted effort has been centered in this direction. Thompson (1917) and Huxley (1932) have contributed greatly toward the bringing together of isolated contributions in this field and they have successfully demonstrated the validity of certain wide empirical generalizations governing many of the known cases of differential growth. The taxonomic importance of structural variations in crabs of the genus *Callinectes* is aptly expressed by Rathbun (1896)—"These variations in the length and form of the appendages are accompanied by other differences, such as shape and sculpture of the carapace, . . . the length of the lateral spine, . . . the form of the abdomen in both sexes. These differences are specific."

The dimensions studied are width, length, distance from the first anterio-lateral serration of the carapace to the lateral carapace spine and the length of the propodite of the right chela (Figure 1). The extent of the size variation of these dimensions is shown graphically and the relation between the magnitudes of the four variables is shown mathematically and represented graphically in Figures 2, 3, 4, 5, 6, 7, and 8. The relations that obtain in males and females are treated separately throughout, and, where possible, biological explanations are offered for the demonstrated variations (Newcombe, 1936).

In presenting this paper, acknowledgment is made to Professor R. V. Truitt, Director of the Chesapeake Biological Laboratory, for the provision of field equipment and laboratory facilities essential to the completion of the problem.

## MATERIALS

The Chesapeake Bay constitutes the center of the distribution of *Callinectes sapidus*. It ranges from Cape Cod to the northern coast of South America. Facts pertaining to its distribution within the Bay throw light on the character and frequencies of the size groups obtained in the collections, all of which were made in the Solomons Island region. Studies on the migration of this crab by Dr. R. V. Truitt[1] have conclusively shown (1) that the crabs of this region are

* Contribution No. 20.

[1] Personal communication.

restricted to the Chesapeake Bay in their distribution, (2) that the smaller crabs move northward toward fresher waters of the Bay until maturity is reached, whereupon the females migrate back to the more saline waters at the mouth of the Bay. These findings indicate that our material collected at random throughout the summer is not representative of a local population and thereby subject to modifications of a local environment, but rather representative of the entire Chesapeake area. The fact that spawning takes place in the southern part of the Bay (Solomons Island occupies a mid position on the

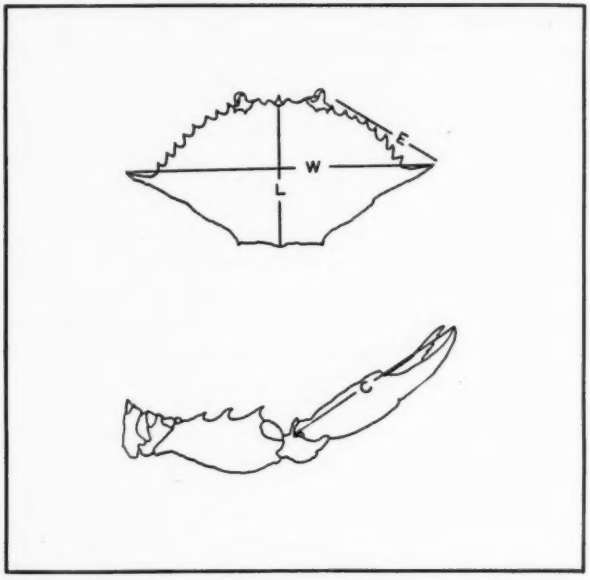


FIG. 1. Outline of *Callinectes sapidus* showing dimensions measured. W=width; L=length; E=eye to spine; C=propodite of the chela.

Chesapeake Bay) accounts for the paucity of material below 30 mm. in width. Furthermore, variations in the abundance and distribution of males and females have presented additional difficulties in securing comparable samples.

Linear measurements were made of specimens ranging in width from 30 mm. to 205 mm. These were carried out during the months of July and August in the years 1935 and 1936. At these times the local air temperatures varied from about 25° to 33° C while the water temperatures near shore ranged from about 25° to 30° C. The water of the region is brackish, the salinity being approximately $11_0/^{00}$ at the surface.

## METHODS

The following linear dimensions have been considered—*width* (W), referring

to the shortest distance between the ends of the lateral spines of the carapace; *length* (L), meaning the perpendicular distance across the carapace from a point immediately posterior to the rostrum to a point just above the first segment of the apron; *eye to spine* (E), implying the distance between the first anterio-lateral serration posterior to the eye and the tip of the large lateral carapace spine (on right side); and *propodite of the right chela* (C), represented by its maximum length (Figure 1). Measurements were made with a vernier caliper reading to the nearest tenth of a millimeter. Except for special purposes, width has been accepted as a basic index of size. As is shown later, this dimension is not necessarily the best index of size for acceptance as a standard.

In all instances the material was grouped in 10 mm. intervals. The respective means and their mathematical constants have been derived, thus giving an expression of the reliability of the differential growth ratios presented here. Constants for the equations,

(1) $L = a + bW$

(2) $E = c + dW$

(3) $C = e + fW$

(4) $E = g + hL$

expressing the relative growth of the four parts, have been obtained by the method of Lipka (1918). In comparing the growth relationships of males and females, length has, in some instances, been used as a basic index of size.

## RESULTS

Individual variations in the linear dimensions L, W, and E are not great (Table 3). The group possessing a mean width of 35.6 mm. shows a standard deviation of 2.8, while the largest group having a mean width of 174.7 mm. has a standard deviation of only 3.0. The standard deviations for the means of "L" and "E" values are in most instances less than those for "W."

All ratios exhibit important differences between the male and female specimens except those for "E" and "W" values. Considering the width-length relations in the male crabs, it is shown that a constant difference applies throughout, the relationship being expressed by the equation $L=4.52+0.412$ W (Table 4). There is a rather large departure in the largest group which suggests the possibility of a significant deviation (Figure 2). In the female specimens, there is not a constant differential growth throughout. Two entirely separate trends obtain, exhibiting a high degree of constancy (Figure 2). The relationship in the lower group is expressed by the equation $L=4.438+0.408$ W in contrast to that for the upper group, namely $L=7.111+0.370$ W. This clearly shows that in the width range below about 140 mm., there is no significant sex variation. Above this size, an entirely different trends is followed in the case of the female specimens. Corresponding absolute increments of length and width (males) bear a constant ratio of 1 to 0.41 throughout the range studied. In the females, this ratio is quite the same up to a width of about 140 mm. when it changes to a 1 to 0.37 ratio. The actual ratio L/W is not constant but decreases

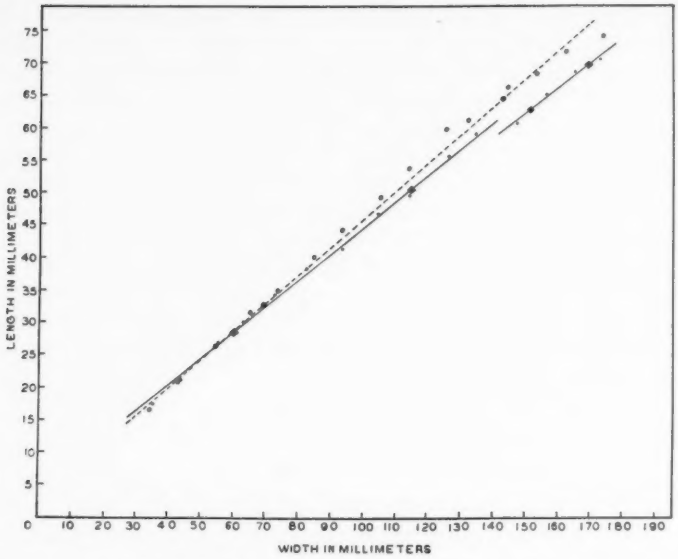


FIG. 2. A comparison of the length-width relations of male and female specimens of *Callinectes sapidus*. L=4.438+0.408 W (lower group), L=7.111+0.370 W (upper group) females; L=4.52+0.412 W (males). Dotted line=males, and continuous line, female specimens. Means of the several groups are plotted.

from 0.524 in male specimens having a width of 40 mm. to 0.438 in crabs 140 mm. wide. This ratio in the females is not significantly different.

At this point reference should be made to the line of best fit shown in Figure 2.

TABLE 1

SIZE OF PARTS IN MILLIMETERS OF *Callinectes sapidus*

| | Males | | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | Range in mm. | No. | Mean of "W" | Mean of "L" | Mean of "E" | No. | Mean of "W" | Mean of "L" | Mean of "E" |
| 1 | 30– 39.9 | 16 | 35.55 | 17.68 | 13.19 | 19 | 36.60 | 18.37 | 13.44 |
| 2 | 40– 49.9 | 27 | 45.26 | 22.40 | 16.95 | 59 | 45.34 | 22.48 | 16.93 |
| 3 | 50– 59.9 | 32 | 54.49 | 26.50 | 20.41 | 83 | 55.29 | 27.05 | 20.76 |
| 4 | 60– 69.9 | 35 | 65.39 | 31.72 | 24.54 | 103 | 64.95 | 31.13 | 24.49 |
| 5 | 70– 79.9 | 31 | 74.28 | 35.29 | 28.35 | 79 | 74.48 | 35.10 | 28.43 |
| 6 | 80– 89.9 | 37 | 85.35 | 40.12 | 33.51 | 65 | 84.30 | 39.19 | 32.34 |
| 7 | 90– 99.9 | 39 | 93.71 | 44.21 | 36.75 | 58 | 94.34 | 42.60 | 36.17 |
| 8 | 100–109.9 | 38 | 104.61 | 48.25 | 41.83 | 38 | 104.66 | 47.25 | 40.87 |
| 9 | 110–119.9 | 37 | 115.31 | 52.71 | 45.63 | 32 | 113.76 | 50.13 | 44.51 |
| 10 | 120–129.9 | 32 | 125.29 | 57.91 | 51.20 | 34 | 124.72 | 55.44 | 50.00 |
| 11 | 130–139.9 | 34 | 134.37 | 60.22 | 53.60 | 25 | 132.71 | 58.98 | 54.22 |
| 12 | 140–149.9 | 42 | 145.72 | 65.35 | 59.08 | 21 | 145.34 | 60.59 | 59.48 |
| 13 | 150–159.9 | 44 | 154.49 | 67.77 | 62.49 | 36 | 155.10 | 64.82 | 64.62 |
| 14 | 160–169.9 | 34 | 163.71 | 71.42 | 66.49 | 37 | 165.12 | 68.51 | 69.20 |
| 15 | 170–179.9 | 24 | 174.68 | 78.97 | 69.22 | 30 | 172.71 | 70.74 | 71.27 |

Its suitability for expressing the facts as we have found them to be is perhaps shown better in Table 2. The fairly close agreement herein revealed seems to constitute a reliable basis for understanding the way in which the dimensions increase throughout the period of life represented by the material. The nature of the relationship between the variables is shown by the mathematical expression $L = 7.111 + 0.370$ W. Using this equation as an illustration we may enquire into the significance that one of this form possesses. The standard deviation about the line of average relationship has been termed the standard

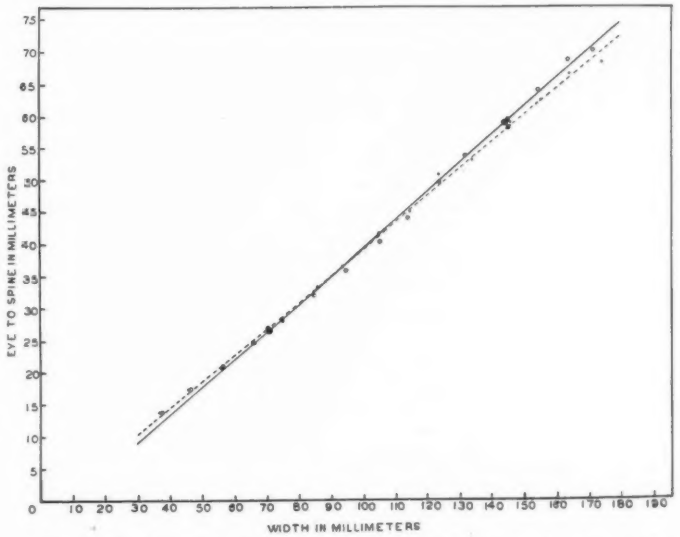


FIG. 3. Showing eye to spine-width relations of *Callinectes sapidus*. $E = -2.213 + 0.4176$ W (males); $E = -3.836 + 0.4360$ W (females). Dotted line = males and continuous line = female specimens. Mean values for the several groups are plotted.

error of estimate (S). The standard error of the L variable $S_l$, calculated from the equation $S_l = \sqrt{\Sigma(d^2)/N}$, has been found to be 1.034, thus indicating a very small degree of dispersion about the line. The significance of the equation may be illustrated further from the coefficient of correlation. Thus, $r = \sqrt{1 - (S_l^2/\sigma_l^2)} = +0.998$. It appears, therefore, that a high degree of confidence may be placed in the afore-mentioned equation used to express the assumed underlying law connecting the linear variables discussed in this paper.

The relation of "E" to "W" is given in Table 4 (Figure 3). It is seen that no significant difference exists between the male and female specimens. Constants for the equation $E = g + hL$ are presented in Table 4. The relation of "E" to "L" appears to be constant throughout life in the males and not significantly different from that of the female specimens below 140 mm. in width (Figure 3). In the upper range, the relation for female crabs is expressed

by the equation $E = -12.155 + 1.1835$ L. In equations (4), (5), (8), (9) and (10), it is shown that "W" varies more than "L" as far as the two sexes are concerned. The sex variations shown by equations (8), (9), and (10), are due to the "E" values which vary in accordance with the widths as is shown in equations (4) and (5).

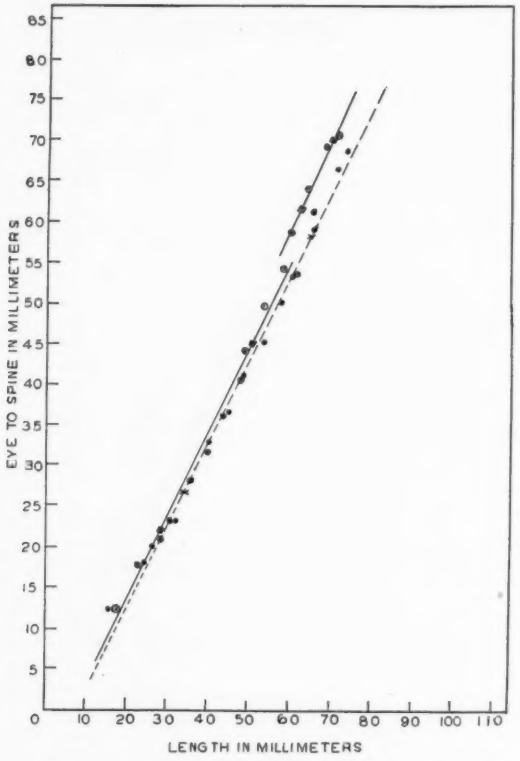


FIG. 4. Showing eye to spine-length relations of *Callinectes sapidus*. $E = -6.72 + 1.0195$ L (lower group); $E = -12.155 + 1.835$ L (upper group) females; $E = -6.782 + 1.0136$ L, males. Dotted line = males, and continuous line = female specimens. Mean values for the several groups are plotted.

The relation between the length of the propodite of the chela (C) and the body width (W) is probably constant in both males and females throughout life but is quite dissimilar in character (Table 4). With increase in width, the "C" value becomes proportionately larger in the male specimens (Figures 7 and 8). Attention is called to the relatively greater individual variations that are found in the size of the propodite of the chela.

TABLE 2

A COMPARISON OF WIDTH ("W") VALUES WITH ACTUAL AND CALCULATED LENGTH ("L") VALUES IN MILLIMETERS OF *Callinectus sapidus*

| Mean "W" | Actual Mean "L" | Cal. Mean "L" | Difference "d" |
|---|---|---|---|
| 35.55 | 17.68 | 19.146 | +1.47 |
| 45.26 | 22.40 | 23.147 | +0.75 |
| 54.49 | 26.50 | 26.949 | +0.45 |
| 65.39 | 31.72 | 31.441 | −0.28 |
| 74.28 | 35.29 | 35.103 | −0.19 |
| 85.35 | 40.12 | 39.664 | −0.46 |
| 93.71 | 44.21 | 43.108 | −1.10 |
| 104.61 | 48.25 | 47.599 | −0.65 |
| 115.31 | 52.71 | 52.008 | −0.70 |
| 125.29 | 57.91 | 56.119 | −1.79 |
| 134.37 | 60.22 | 59.860 | −0.36 |
| 145.72 | 65.35 | 64.536 | −0.81 |
| 154.49 | 67.77 | 68.149 | +0.38 |
| 163.71 | 71.42 | 71.948 | +0.53 |
| 174.68 | 73.97 | 76.468 | +2.50 |

## DISCUSSION

Hay (1905) and Churchill (1917) in their studies of the life history of the blue crab made a few width measurements of both male and female specimens. No attempt appears to have been made to evaluate the degree of variation and the character of linear growth dimensional ratios in this form. Huxley (1932) gives a comprehensive review of the literature dealing with the relative growth rates

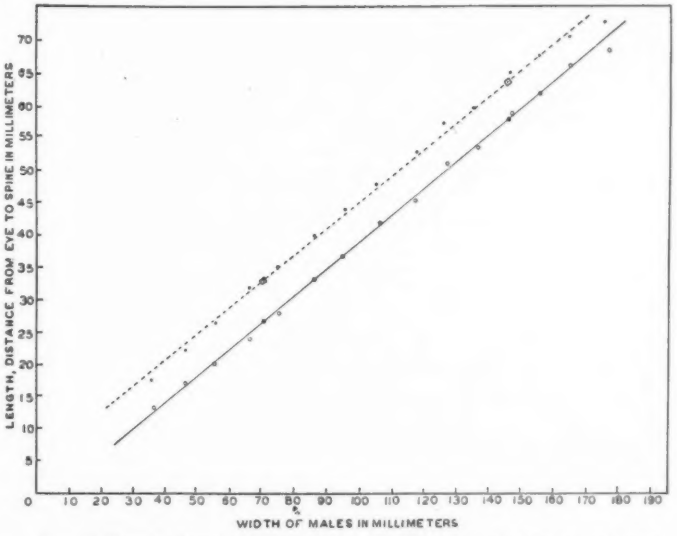


FIG. 5. Showing length-width and eye to spine-width relations in *male* specimens of *Callinectes sapidus*. L=4.52+0.412 W; E=−2.213+0.4176 W. Dotted line=length, and continuous line=eye to spine dimension.

of different organs in a variety of animals. He provides evidence to show that the simple heterogony formula $L = xW^k$, or an approximation to it, holds for a number of organs. Reference is made to several species of prawns and crabs in which the linear dimensions studied exhibit constant differential growth ratios (Compare Newcombe and Kessler, 1936; Phillips and Newcombe, 1937; Newcombe and Gould, 1937.). Richards (1935) has made a critical examination of

TABLE 3

CONSTANTS OF FREQUENCY DISTRIBUTIONS OF "W," "L" AND "E" VALUES IN MILLIMETERS OF *Callinectes sapidus*

| Group | No. | Range | "W" Values | | "L" Values | | "E" Values | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | Stand. dev. | Mean | Stand. dev. | Mean | Stand. dev. |
| | | | Males | | | | | |
| 1 | 16 | 30– 39.9 | 35.6±0.5 | 2.8±0.3 | 17.7±0.3 | 1.7±0.2 | 13.2±0.2 | 1.2±0.2 |
| 2 | 27 | 40– 49.9 | 45.3±0.3 | 2.1±0.2 | 22.4±0.2 | 1.4±0.1 | 17.0±0.2 | 1.8±0.2 |
| 3 | 32 | 50– 59.9 | 54.5±0.3 | 2.4±0.2 | 26.5±0.2 | 1.6±0.1 | 20.4±0.2 | 1.7±0.1 |
| 4 | 35 | 60– 69.9 | 65.4±0.4 | 3.0±0.2 | 31.7±0.2 | 1.9±0.2 | 24.5±0.2 | 1.5±0.1 |
| 5 | 31 | 70– 79.9 | 74.3±0.3 | 2.7±0.2 | 35.3±0.3 | 2.1±0.2 | 28.4±0.2 | 1.9±0.2 |
| 6 | 37 | 80– 89.9 | 85.4±0.3 | 2.4±0.2 | 40.1±0.3 | 2.2±0.2 | 33.5±0.2 | 1.8±0.2 |
| 7 | 39 | 90– 99.9 | 93.7±0.3 | 3.0±0.2 | 44.2±0.3 | 2.7±0.2 | 36.8±0.2 | 2.2±0.2 |
| 8 | 38 | 100–109.9 | 104.6±0.2 | 2.1±0.2 | 48.3±0.4 | 3.8±0.3 | 41.8±0.2 | 1.9±0.2 |
| 9 | 37 | 110–119.9 | 115.3±0.3 | 3.1±0.2 | 52.7±0.3 | 2.6±0.2 | 45.6±0.2 | 1.8±0.1 |
| 10 | 32 | 120–129.9 | 125.3±0.4 | 3.2±0.3 | 57.9±0.4 | 3.4±0.3 | 51.2±0.5 | 4.0±0.3 |
| 11 | 35 | 130–139.9 | 134.4±0.5 | 4.3±0.3 | 60.2±0.6 | 4.9±0.4 | 53.6±0.3 | 2.5±0.2 |
| 12 | 42 | 140–149.9 | 145.7±0.4 | 3.3±0.2 | 65.4±0.2 | 2.0±0.2 | 59.1±0.3 | 2.9±0.2 |
| 13 | 44 | 150–159.9 | 154.5±0.3 | 3.1±0.2 | 67.8±0.3 | 2.6±0.2 | 62.5±0.2 | 2.2±0.2 |
| 14 | 34 | 160–169.9 | 163.7±0.4 | 3.2±0.3 | 71.4±0.3 | 2.7±0.2 | 66.5±0.3 | 2.2±0.2 |
| 15 | 24 | 170–179.9 | 174.7±0.4 | 3.0±0.3 | 74.0±0.5 | 3.3±0.3 | 69.2±0.3 | 2.1±0.2 |
| | | | Females | | | | | |
| 1 | 19 | 30– 39.9 | 36.6±0.4 | 2.5±0.3 | 18.4±0.3 | 1.8±0.2 | 13.4±0.2 | 1.5±0.2 |
| 2 | 50 | 40– 49.9 | 45.3±0.3 | 2.9±0.2 | 22.5±0.2 | 1.9±0.1 | 16.9±0.2 | 1.9±0.1 |
| 3 | 83 | 50– 59.9 | 55.3±0.2 | 2.9±0.2 | 27.1±0.1 | 1.5±0.1 | 20.8±0.1 | 1.7±0.1 |
| 4 | 103 | 60– 69.9 | 64.6±0.2 | 3.1±0.2 | 31.1±0.1 | 1.9±0.1 | 24.5±0.2 | 2.3±0.1 |
| 5 | 79 | 70– 79.9 | 74.5±0.2 | 2.9±0.2 | 35.1±0.2 | 2.4±0.1 | 28.4±0.2 | 2.1±0.1 |
| 6 | 65 | 80– 89.9 | 84.3±0.2 | 2.8±0.2 | 39.2±0.2 | 2.1±0.1 | 32.3±0.2 | 2.0±0.1 |
| 7 | 58 | 90– 99.9 | 94.3±0.2 | 2.5±0.2 | 42.6±0.4 | 4.4±0.3 | 36.2±0.2 | 2.3±0.2 |
| 8 | 38 | 100–109.9 | 104.7±0.3 | 3.1±0.2 | 47.3±0.1 | 1.2±0.1 | 40.9±0.5 | 4.3±0.3 |
| 9 | 32 | 110–119.9 | 113.8±0.3 | 2.3±0.2 | 50.1±0.3 | 2.3±0.2 | 44.5±0.2 | 2.0±0.2 |
| 10 | 34 | 120–129.9 | 124.7±0.3 | 2.8±0.3 | 55.4±0.3 | 2.8±0.2 | 50.0±0.2 | 1.8±0.2 |
| 11 | 25 | 130–139.9 | 132.7±0.4 | 2.8±0.3 | 59.0±0.4 | 3.1±0.3 | 54.2±0.5 | 3.6±0.3 |
| 12 | 21 | 140–149.9 | 145.3±0.4 | 2.8±0.3 | 60.6±0.5 | 3.2±0.3 | 59.5±0.8 | 5.4±0.6 |
| 13 | 36 | 150–159.9 | 155.1±0.2 | 2.7±0.2 | 64.8±0.3 | 2.8±0.2 | 64.6±0.2 | 2.0±0.2 |
| 14 | 37 | 160–169.9 | 165.1±0.4 | 3.2±0.3 | 68.5±0.4 | 3.1±0.2 | 69.2±0.4 | 3.1±0.2 |
| 15 | 30 | 170–179.9 | 172.7±0.3 | 2.7±0.2 | 70.7±0.3 | 2.1±0.2 | 71.3±0.3 | 2.4±0.2 |

the use of the heterogonic constant $k$ in the formula $L = xW^k$ used for fitting the growth of a part of an organism with respect to that of the rest of the animal. He advances theoretical as well as practical objections to the concept of a constant partition of materials between the part and the whole organism.

It is shown in Figures 2–7 that the equation $L = a + bW$ gives a fairly accurate fit and seems to express quite clearly the observations made (Table 2). The variables considered here increase in fairly close approximation to a constant differential growth rate. In the case of the females, the slope changes at a width of about 140 mm. and the probable reason for this is discussed below.

Considering first the individual variations that exist in the variables compared, it should be pointed out that they are not of the same order of magnitude. This is not expected in an animal which undergoes so many moults. Furthermore, this variation is increased in the propodite measurements of the chela since this appendage is often lost and not completely regenerated until after two or more moults (Figures 7 and 8.) By comparing both chelae when the measurements were made, it was usually possible to eliminate the most significant variations produced by incomplete regeneration. The variations found in the other variables are not unusually high as shown in Table 3.

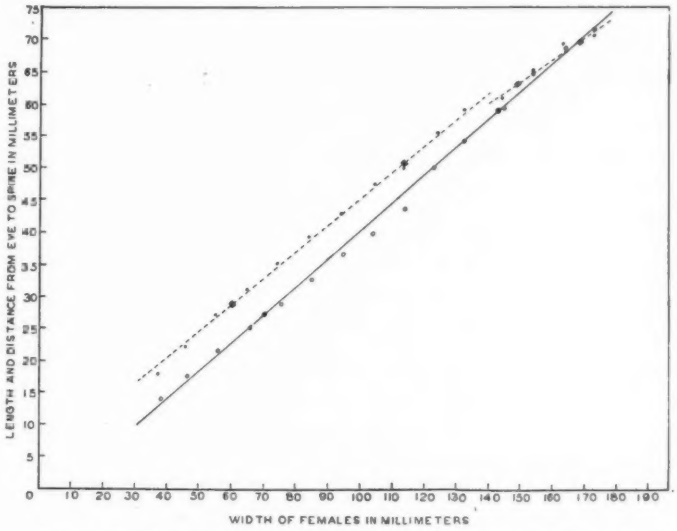


FIG. 6. Showing length-width and eye to spine-width relations in *female* specimens of *Callinectes sapidus*. L=4.438+0.408 W (lower group); L=7.111 +0.370 W (upper group); E=−3.836+0.4360 W. Dotted line=length, and continuous line=eye to spine dimension.

Variation in the width-length relations between males and females is very marked as shown in equations (1), (2), and (3) of Table 4 (Figure 2). Comparing "W" and "E" values, no sex variation is found whereas the "L"–"E" ratios show a decided variation between males and females. The explanation seems to lie in the fact that length is the dimension least susceptible to sex variation. Observations have shown that at sizes above 140 mm. in width, the lateral carapace spines of the female specimens straighten out and become relatively longer than in the males. Since the "E" variable is dependent on these lateral spines, it is not to be expected that the relations of "E" to "W" would be appreciably affected (Table 4). This assumption is confirmed by the change in the slope of the curve above a width of about 140 mm. in the "E"–"L" ratios of the females (Figure 4). This break in the curve is due to a sudden

TABLE 4

EQUATIONS EXPRESSING THE GROWTH RELATIONS OF THE PARTS OF *Callinectes sapidus*

| No. | Sex | Group | Equation |
|---|---|---|---|
| 1 | Male | | L= 4.52 +0.412 W |
| 2 | Female | Lower group | L= 4.438+0.408 W |
| 3 | Female | Upper group | L= 7.111+0.370 W |
| 4 | Male | | E= − 2.213+0.4176 W |
| 5 | Female | | E= − 3.836+0.4360 W |
| 6 | Male | | C= −10.805+0.6246 W |
| 7 | Female | | C= 1.982+0.4356 W |
| 8 | Male | | E= − 6.782+1.0136 L |
| 9 | Female | Lower group | E= − 6.72 +1.0195 L |
| 10 | Female | Upper group | E= −12.155+1.1835 L |

increase of the "E" dimension without a corresponding increase in the "L" variable. Field observations clearly show that the lateral carapace spines are longer in large females than in males of corresponding length. The accompanying figures show that the change begins when a width of about 140 mm. is attained.

The trend of growth followed by the "E" and "L" variables when compared with width as a base is shown in Figure 5 for the males and in Figure 6 for females. In general, the slopes are quite similar throughout except where the ratio definitely changes in the female crabs and to a less extent in the male crabs. The long straight spines of the female are indicative of the last moult when sexual maturity is reached. A much less conspicuous carapace variation is shown at this stage in the males. Their lateral spines increase but slightly and this increment is minimized by a small terminal curvature. The deviation in the case of the last group of males (Figure 2) may also be a result of sexual maturity, although there is no definite external character in the male to indi-

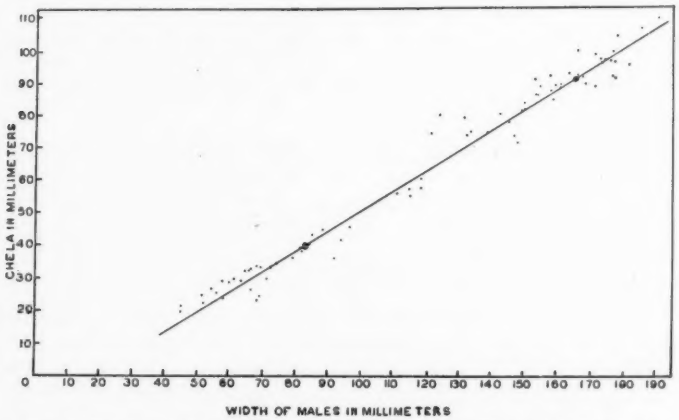


FIG. 7. Showing chela-width relations of *Callinectes sapidus* (male specimens); C= −10.805+0.6246 W.

cate when this stage is reached. This assumption is supported by the fact that a line projecting back from the last group intersects the line of best fit for the lower group near 140 mm., the width at which maturity in the female may be recognized by other definite characters. It would seem, therefore, that some physiological change in the maturing crab operates to modify the character of the exoskeleton, being most apparent in the females. How general this tendency may be in respect to other skeletal parts is still a matter of conjecture. The validity of length as a basic index of size is pointed out and should, for many purposes, be considered superior to the other variables treated here.

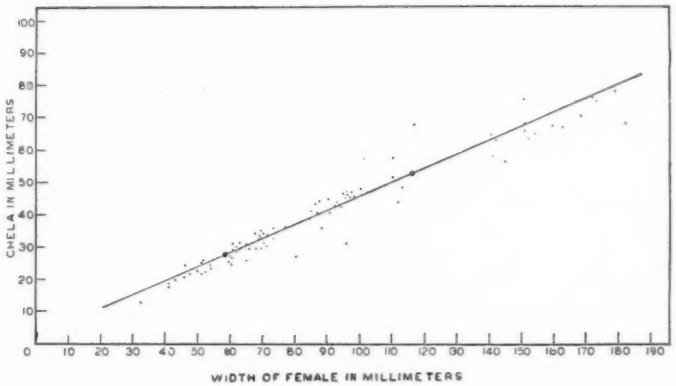


FIG. 8. Showing chela-width relations of *Callinectes sapidus* (female specimens); C=1.982+0.4356 W.

The relation between the "C" and "W" values for the males and females is shown in Figures 7 and 8. The data are insufficient to give a conclusive picture of the existing ratios. They do indicate, however, the great variability in size of the chela and the marked difference between the male and female specimens (Table 4). Field observations lead us to conclude that a principal source of variation is found in the frequent loss of the chela during moulting, the normal size of which is not regained before the third succeeding moult (Churchill, 1917, p. 107).

## SUMMARY AND CONCLUSION

Four linear dimensions of the Chesapeake blue crab, *Callinectes sapidus*, have been studied from the following viewpoints: (1) variation, (2) sex differences, and (3) quantitative variations in differential growth rates. Collections were made in the region of Solomons Island during July and August of the years 1935 and 1936.

It is concluded that length constitutes a better standard index of size than either of the other three variables examined.

Individual variations in "W," "L," and "E" values are not excessive.

A constant differential growth rate is followed in respect to the four variables studied. At a width of about 140 mm., the theoretical average size at which sexual maturity appears to be reached, a new relationship is established that remains fairly constant.

In the width range below 140 mm., a close parallelism exists between the males and the females with respect to the "WL" and "LE" relations (Table 4). Above this point, marked variation occurs. In the case of the females, two distinct relations obtain when "L" is compared with the "W" and "E" variables. The break occurs at about the 140 mm. point, the average width at which they become mature. In male specimens, increase in width is accompanied by a proportionate increase in the other dimensions.

Length is the most constant variable since both "W" and "E" are influenced by a sudden elongation of the lateral carapace spines at maturity and "C" is often affected by abnormal growth due to injury.

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# SOME PHYSIOLOGICAL PHENOMENA FITTED TO GROWTH EQUATIONS

## III. RATE OF GROWTH OF BRAIN POTENTIALS (ALPHA FREQUENCY) COMPARED WITH RATE OF GROWTH OF THE BRAIN

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(*Received September 8, 1938*)

In the second paper of this series (1) the increase in frequency with age of the brain potentials (alpha frequency or Berger rhythm) was fitted to the exponential growth equation

$$f = f_m - f_m e^{-k(A-A')} \tag{1}$$

where $f$ is the frequency of the alpha waves in oscillations per second; $A$ is the age of the individual in years; $f_m$ is the maximum frequency attained in adult life and $k$ is the time-rate of decline in frequency ($100k$ is the percentage rate) or the velocity constant of growth in frequency of the brain potentials. $A'$ is an age parameter.

Much credit goes to Dr. Samuel Brody of the Missouri Agricultural Experiment Station, who has extensively used expressions similar to equation (1) in evaluating rates of growth, mature weights, equivalence of age, etc., of various animals, man, plants, bacteria and populations, in body weight, height, size and number for that phase of growth following puberty or that phase of growth following the inflection point in the growth curve, and termed self-inhibiting growth by Dr. Brody (2).

Dr. Brody has carefully derived this expression for self-inhibiting growth on the basis of instantaneous (true) growth rates, and he has rationalized and derived the expression as an extension of the physico-chemical principle of mass action (3).

In this paper the growth in the weight of the brain from data collected by Vierordt quoted from Donaldson (4) is fitted to an equation similar to equation (1)

$$W = W_m - W_m e^{-k(A-A')} \tag{2}$$

and the velocity of growth in brain weight compared with the velocity of growth in frequency of the brain potentials.

The data of Lindsley (5) and Smith (6) for the frequency of the alpha waves at different ages in the human is reproduced from the second paper in this series in Fig. 1b. The value of $k$, the time-rate of decline in frequency, is 0.468 or 46.8 per cent per year as determined in the second paper of this series.

Fig. 1a is a plot of Vierordt's data (4) on the total weight of the brain as ordinates versus the age as abscissae. A total of 839 brains were used for this data, 415 males and 424 females, with an average of 38 to 39 brains for each age to

7 years. From 7 years on the data for the weight of the brain is fairly constant, so that all brain weights from 7 to 25 years of age, both male and female, were averaged together to give the mature weight of the adult brain, $W_m$, 1333 grams.

The velocity of growth of the total weight of the brain is obtained from the slope of the semi-logarithmic plot, $1n\ (W_m - W)$ versus $A$ as illustrated in Fig. 2. $A'$ is read from the graph as that age where the curve crosses the age-axis, or that age where the extrapolated semi-log plot reaches the maximum or mature weight, $W_m$, 1333 grams.

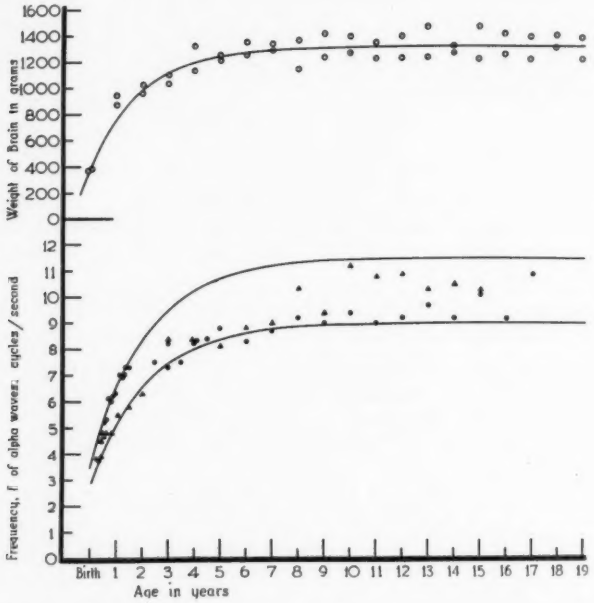


FIG. 1a (top). Increase in the weight of the brain in grams as ordinates versus age as abscissae.
FIG. 1b (bottom). Increase in the frequency of the brain potentials (alpha waves) as ordinates versus age as abscissae.

The value of $k$ for the increase in the weight of the brain is 0.485 or 48.5 per cent per year. This value is very close to that for the velocity of growth of the brain potentials. $A'$ is $-0.70$.

The growth of the brain in weight and the growth of the frequency of the Berger rhythm follow a similar course and from the values of the velocity constants of growth are seen to grow at approximately the same rate. To further illustrate this point, an equivalence chart was constructed (see Fig. 3) showing the relation between brain-weight age and brain-potential age. These charts and their preparation are fully explained by Brody (7). Briefly, the essential point in constructing an equivalence chart is to plot the per cent of the mature

value reached at a given age versus $k(A-A')$ as abscissae. The appropriate age scales are then laid off.

Fig. 3 illustrates the high degree of correlation between brain weight and the frequency of brain potentials at a given age. It is noted that the age when a given fraction of the mature brain weight is reached very nearly corresponds with the age when the same fraction of the mature frequency of the brain potentials is reached.

The relation between the weight of the brain and the frequency of the alpha

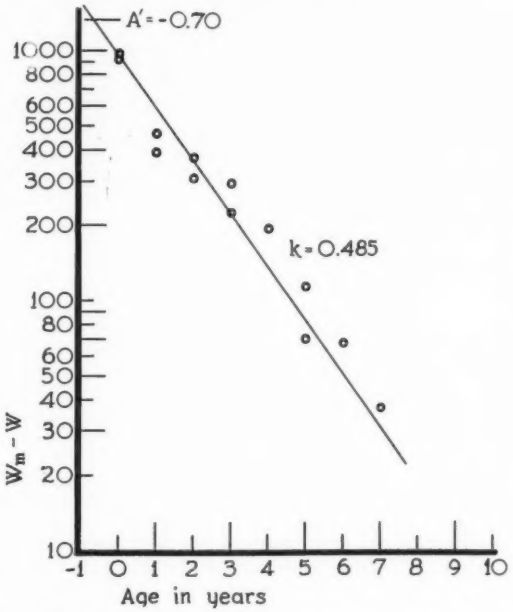


FIG. 2. Semilogarithmic plot of $W_m - W$ versus age giving the velocity of growth of the brain as the slope of the straight line.

waves may be obtained by eliminating $A$ from equations (1) and (2). However, from the equivalence chart,

$$\frac{W}{W_m} = \frac{f}{f_m} \tag{3}$$

is nearly true. Then

$$W = f\frac{W_m}{f_m}. \tag{3a}$$

Taking the mature frequency of the alpha waves as 10 and the mature weight of the brain as the average of the males and females of Vierordt's data, 1333

grams, we have

(3b) $$W = 133.3 \cdot f.$$

Equation (3b) gives an approximate method of predicting the size of the growing brain from measurements of the frequency of the alpha waves.

When two different phenomenon have the same type of secular trend it is obvious that they will correlate closely with each other. It does not follow from this alone that the two phenomena are dependent upon each other or in any way connected.

Here we have brain potentials and the weight of the brain, both increasing

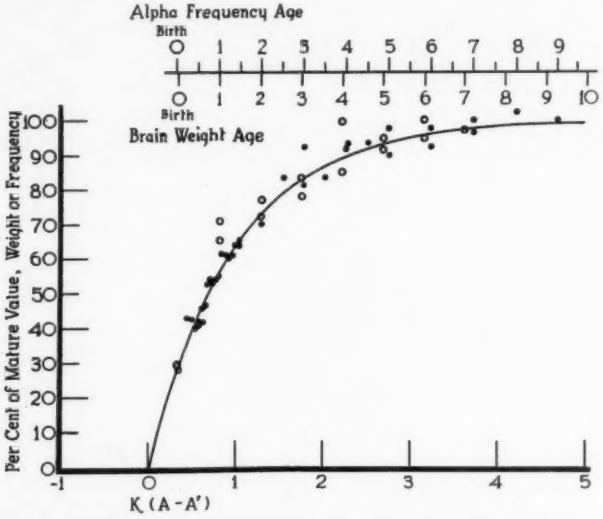
Alpha Frequency Age
Birth
0 1 2 3 4 5 6 7 8 9
0 1 2 3 4 5 6 7 8 9 10
Birth
Brain Weight Age
Per Cent of Mature Value, Weight or Frequency
100 90 80 70 60 50 40 30 20 10 0
-1 0 1 2 3 4 5
K (A - A')

FIG. 3. Equivalence of age between the growth of the brain potentials (alpha frequency) in solid circles and the growth of the weight of the brain in open circles. Note that the age scales are almost together and that when a given fraction of the mature weight of the brain is reached approximately the same fraction of the mature frequency of the brain potentials is reached.

in magnitude with time. That they should correlate is natural, but we can only, at this time, suggest a possible relationship from the preceding analysis, a relationship which seems likely because of our axiomatic premise that there is something functional between the *brain* and its *electrical manifestations*. That they both should increase in magnitude at approximately the same rate suggests that the weight of the brain which may be thought of as a measure of cell number or cell size may be directly responsible for or proportional to the frequency of the brain potentials. Since the Berger rhythm is understood to be a manifestation or result of occipital lobe activity, the comparison should be made between the frequency of the rhythm and the weight of the occipital lobes. It is possible that the occipital lobes grow at the same rate as does the entire brain.

## SUMMARY

The rate of growth of the weight of the brain was determined, compared with the rate of growth of the brain potentials (alpha frequency, or Berger rhythm) and found to be approximately the same.

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# MINERAL GROWTH

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(*Received October 3, 1938*)

Huxley (3) in 1924 first considered the relation between the growth of parts of a living organism when the parts were increasing or decreasing in size. The process is described by the equation: $\log y = \log b + k \log x$, where $y$ is the size of the part, $x$ the size of the whole minus the size of $y$, $b$ the constant representing the fraction of $x$ which $y$ occupies when $x$ is unity, and $k$, the ratio of the growth rate of the part to the growth rate of the whole. The constant $b$ has little meaning, the constant $k$ on the other hand has great biological significance.

By the method of least squares, this simple relationship is applied to the mineral composition of the human fetus and the very young rat. The plotting of chemical entity against chemical whole as represented by dry weight or water-fat free weight on a double logarithmic scale gives without exception a straight line. The fetal data used in the calculations was published in 1934 (4), the rat data is summarized in Tables I and II. The data of Table I is supplemented by a further series of rats given in Table II. Since the latter series seemed heavier in weight, all calculations were made on the basis of water-fat free weight as well as dry weight. The data is unweighted, since errors in observation seemed to warrant neglecting the weights.

TABLE I

THE CHEMICAL COMPOSITION OF THE YOUNG RAT

| Rats | Age Days | Moist Weight gm. | Dry Weight gm. | Water per cent | Fat per cent | Calcium mgm. | Phosphorus mgm. | Nitrogen mgm. | Potassium mgm. | Sodium mgm. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Newborn | 5.359 | 0.708 | 86.8 | 1.2 | 12.1 | 12.8 | 82.8 | 11.2 | 9.4 |
| | 1 | 5.709 | 0.927 | 83.8 | 2.8 | 14.5 | 16.1 | 93.0 | 13.0 | 10.5 |
| | 2 | 6.509 | 1.092 | 83.2 | 3.4 | 19.1 | 20.7 | 112.8 | 16.0 | 11.3 |
| | 3 | 6.599 | 1.118 | 83.1 | 3.9 | 19.2 | 20.7 | 117.8 | 16.7 | 11.3 |
| | 4 | 8.036 | 1.425 | 82.3 | 4.2 | 23.2 | 23.1 | 146.0 | 20.5 | 12.5 |
| | 5 | 9.359 | 1.809 | 80.7 | 4.9 | 30.6 | 28.1 | 182.0 | 22.1 | 14.7 |
| | 6 | 10.055 | 2.002 | 81.0 | 6.0 | 35.0 | 34.3 | 191.0 | 22.5 | 15.3 |
| | 7 | 11.420 | 2.297 | 79.9 | 5.8 | 40.3 | 39.2 | 232.0 | 21.8 | 16.8 |
| | 8 | 12.621 | 2.691 | 78.7 | 7.0 | 47.4 | 49.2 | 241.0 | 25.9 | 19.4 |
| | 10 | 15.773 | 3.729 | 76.4 | 8.0 | 65.0 | 62.1 | 314.0 | 34.3 | 20.9 |
| | 12 | 17.889 | 4.559 | 74.5 | 9.4 | 84.4 | 83.1 | 360.0 | 44.0 | 25.8 |
| | 14 | 22.280 | 6.271 | 71.9 | 12.1 | 99.3 | 109.3 | 469.0 | 54.8 | 29.0 |
| | 16 | 23.633 | 6.814 | 71.1 | 8.8 | 128.6 | 108.0 | 488.0 | 59.9 | 31.8 |
| | 20 | 32.190 | 9.580 | 70.2 | 10.1 | 197.9 | 161.3 | 730.0 | 73.9 | 42.7 |
| | 24 | 51.410 | 14.683 | 71.5 | 8.3 | 334.3 | 274.0 | 1187.0 | 152.0 | 59.7 |

Table III summarizes the statistical data governing the constants $b$ and $k$: the probable error of $k$, the correlation coefficient, $r$, the probable error of $r$,

The authors express their indebtedness to Professor Sewall Wright for suggestions and criticism.

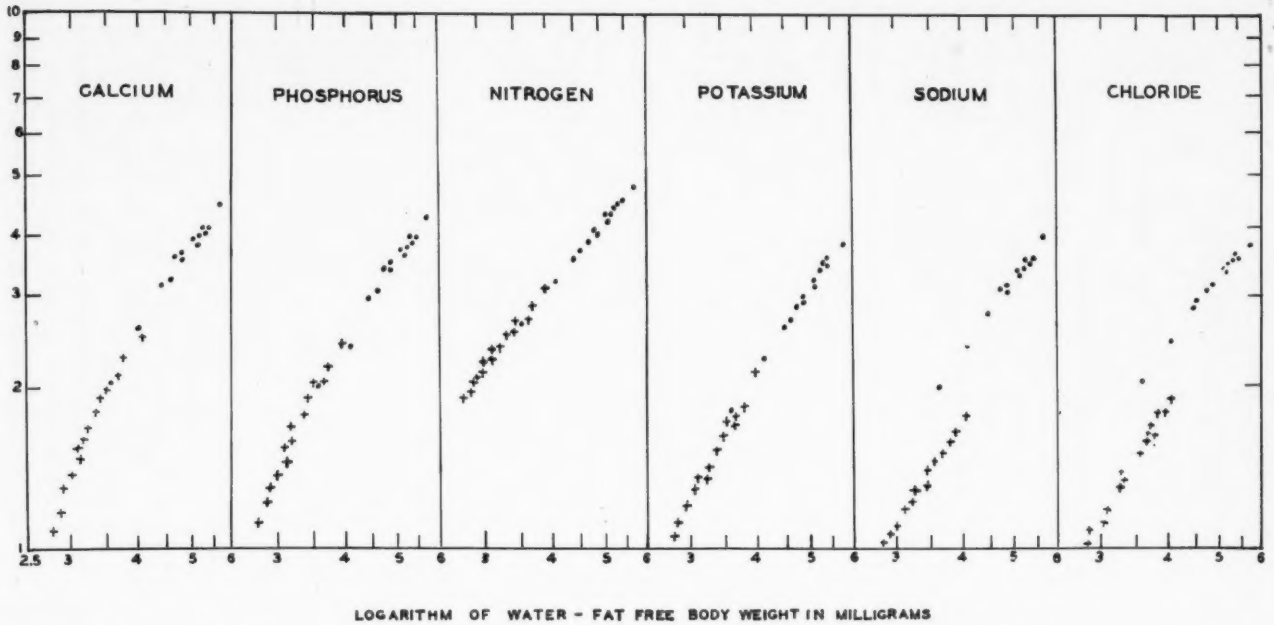


FIG. 1. Relative Growth of Human Fetus and Young Rat. Weight of chemical constituent against water-fat free body weight minus weight of constituent, logarithmic plotting. Fetal data are represented by dots, rat data by crosses, *k*'s for fetus and rat: Ca, 1.08 and 1.19; P, 1.04 and 1.09; N, 1.00 and 0.92; K, 0.91 and 0.86; Na, 0.84 and 0.66; Cl, 0.80 and 0.72.

and the correlation coefficient corrected for fewness of terms according to the correction formula (1): $\bar{r}^2 = 1-(1-r^2)(N-1/N-2)$. On the whole fairly good correlation is obtained, best in the case of nitrogen and poorest in the case of sodium in the rat.

Concerning the growth rates of Table III, it is of interest to note that only the $k$'s of those minerals involved in the controversy of storage, calcium, phosphorus, and iron, are above unity in the fetus. The low $k$ of iron in the rat may

TABLE II

THE IRON AND CHLORIDE CONTENT OF THE YOUNG RAT

| Rat Age | Moist Weight* gm. | Dry Weight** gm. | Water per cent | Fat per cent | Chlorine mgm. | Iron mgm. |
|---|---|---|---|---|---|---|
| Newborn | 5.809 | 0.796 | 86.3 | 1.0 | — | 0.382 |
| Newborn | 5.470 | 0.715 | 86.9 | 0.9 | — | 0.335 |
| Newborn | 5.877 | 0.817 | 86.1 | 1.0 | 12.32 | — |
| Newborn | 5.767 | 0.828 | 85.6 | 1.0 | 12.02 | — |
| 2 Days | 6.385 | 1.020 | 84.0 | 4.1 | — | 0.455 |
| 2 Days | 6.168 | 1.008 | 83.6 | 4.2 | — | 0.417 |
| 2 Days | 5.036 | 0.796 | 84.2 | 4.0 | 9.84 | — |
| 2 Days | 5.340 | 0.826 | 84.5 | 4.0 | 10.47 | — |
| 4 Days | 8.187 | 1.441 | 82.4 | 5.2 | — | 0.450 |
| 4 Days | 8.324 | 1.457 | 82.5 | 5.2 | — | 0.470 |
| 4 Days | 7.775 | 1.646 | 79.0 | 6.3 | 13.67 | — |
| 4 Days | 8.624 | 1.803 | 79.1 | 6.2 | 15.44 | — |
| 6 Days | 11.512 | 2.139 | 81.4 | 5.6 | — | 0.607 |
| 6 Days | 14.151 | 2.885 | 80.0 | 6.2 | — | 0.532 |
| 6 Days | 13.987 | 3.074 | 78.0 | 6.6 | 24.19 | — |
| 6 Days | 13.487 | 2.956 | 78.1 | 6.6 | 24.07 | — |
| 8 Days | 12.384 | 2.805 | 77.4 | 8.6 | 20.56 | 0.591 |
| 8 Days | 13.976 | 3.244 | 76.8 | 7.1 | 22.72 | 0.621 |
| 10 Days | 25.172 | 6.211 | 72.7 | 9.2 | 34.55 | 0.882 |
| 10 Days | 22.476 | 6.268 | 72.1 | 11.3 | 34.19 | 1.001 |
| 12 Days | 28.291 | 8.429 | 70.2 | 11.6 | 41.84 | 1.127 |
| 12 Days | 30.675 | 9.078 | 70.4 | 11.7 | 45.87 | 1.181 |
| 14 Days | 31.420 | 9.374 | 70.2 | 11.6 | 51.04 | 0.977 |
| 14 Days | 30.644 | 9.447 | 69.2 | 12.4 | 45.10 | 0.976 |
| 16 Days | 42.633 | 14.939 | 65.0 | 15.7 | 65.70 | 1.375 |
| 16 Days | 42.400 | 14.768 | 65.2 | 15.3 | 63.20 | 1.541 |
| 20 Days | 49.897 | 18.945 | 66.1 | 18.4 | 65.44 | 1.619 |
| 24 Days | 61.787 | 24.475 | 64.0 | 19.4 | 85.71 | 2.010 |

* After removal of G.I. tract.

** After 48 hours in electric oven at 105°C.

reflect the iron starvation anemia of nurslings. On the basis of water-fat free weight, the fetal nitrogen growth rate equals that of the body, potassium, sodium and chloride range from 10 to 20 per cent lower. The rat shows a similar relationship except for the lower values of sodium and chloride. Figure I, a double logarithmic plot of all the chemical constituents except iron, reveals this exception of sodium and chloride. The difference may possibly be one of species: average Na/Cl ratios in milligrams of fetus (excluding the term value) and of the newborn rat are 0.87 and 0.96 (7) respectively. Sodium and chloride values of the serum of the rat, 134.6 and 104.7 m.-eq. per litre serum (6) may be considered low with respect to sodium when compared with values judged normal for human serum (2).

TABLE III

STATISTICAL DATA OF FETAL AND RAT GROWTH

| Element | log $b$ | $k$ | Probable Error of $k$ | Correlation coef., $r$ | Probable Error of $r$ | Correlation coef. corrected for fewness of terms, $\bar{r}$ |
|---|---|---|---|---|---|---|
| | | | Fetus—Dry Weight | | | |
| Calcium | −1.5707 | 1.024 | 0.028 | 0.990 | 0.004 | 0.989 |
| Phosphorus | −1.5064 | 0.979 | 0.023 | 0.993 | 0.003 | 0.993 |
| Nitrogen | −0.6914 | 0.943 | 0.009 | 0.999 | 0.000 | 0.999 |
| Potassium | −1.2688 | 0.859 | 0.015 | 0.996 | 0.001 | 0.996 |
| Sodium | −0.8393 | 0.799 | 0.014 | 0.996 | 0.001 | 0.996 |
| Chloride | −0.5863 | 0.760 | 0.019 | 0.994 | 0.002 | 0.994 |
| Iron | −3.2526 | 0.967 | 0.024 | 0.994 | 0.002 | 0.993 |
| | | | Fetus—Fat Free Dry Weight | | | |
| Calcium | −1.7819 | 1.084 | 0.025 | 0.993 | 0.003 | 0.992 |
| Phosphorus | −1.7231 | 1.036 | 0.018 | 0.996 | 0.002 | 0.995 |
| Nitrogen | −0.9045 | 1.000 | 0.004 | 1.000 | 0.000 | 1.000 |
| Potassium | −1.4438 | 0.906 | 0.015 | 0.996 | 0.001 | 0.996 |
| Sodium | −0.9971 | 0.841 | 0.011 | 0.998 | 0.001 | 0.998 |
| Chloride | −0.7668 | 0.803 | 0.013 | 0.997 | 0.001 | 0.996 |
| Iron | −3.5985 | 1.049 | 0.028 | 0.991 | 0.003 | 0.990 |
| | | | Rat—Dry Weight | | | |
| Calcium | −2.0129 | 1.079 | 0.014 | 0.998 | 0.001 | 0.997 |
| Phosphorus | −1.7210 | 0.994 | 0.013 | 0.998 | 0.001 | 0.997 |
| Nitrogen | −0.4469 | 0.837 | 0.014 | 0.996 | 0.001 | 0.996 |
| Potassium | −1.1893 | 0.778 | 0.026 | 0.985 | 0.005 | 0.984 |
| Sodium | −0.7859 | 0.614 | 0.026 | 0.976 | 0.009 | 0.974 |
| Chloride | −0.7312 | 0.603 | 0.032 | 0.994 | 0.002 | 0.994 |
| Iron | −1.8228* | 0.479 | 0.010 | 0.990 | 0.003 | 0.989 |
| | | | Rat—Fat Free Dry Weight | | | |
| Calcium | −2.2068 | 1.188 | 0.012 | 0.998 | 0.001 | 0.998 |
| Phosphorus | −1.8870 | 1.087 | 0.022 | 0.994 | 0.002 | 0.994 |
| Nitrogen | −0.5831 | 0.922 | 0.016 | 0.996 | 0.002 | 0.995 |
| Potassium | −1.3312 | 0.857 | 0.023 | 0.990 | 0.004 | 0.989 |
| Sodium | −0.8700 | 0.655 | 0.009 | 0.998 | 0.001 | 0.997 |
| Chloride | −1.0118 | 0.716 | 0.009 | 0.997 | 0.001 | 0.997 |
| Iron | −2.0396* | 0.563 | 0.014 | 0.986 | 0.004 | 0.985 |

* Adjusted values.

With exceptions of sodium, chloride and iron, the growth constants for the human fetus and the young rat are very similar, in spite of great differences in absolute weights and ages. This similarity of chemical development expressed by a system of relations or ratios is offered as further evidence for the "fundamental chemical plan for animal growth" (5).

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# IS COLCHICINE A "PHYTOHORMONE"?

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"Growth is not single but multiple."
F. S. Hammett

The present symposium is limited to the "hormonal" aspects of the action of colchicine in plants. Its object is a contribution to the study of the mechanisms which regulate incremental growth and differentiation and form a connecting link between some of the physiological and pathological aspects of growth.

In the course of investigations on the effects of Colchicine on plants, which were initiated in this Institute in 1936, I have found that some of the responses of plants to treatments with this substance were strikingly similar to those produced by several of the "growth substances," or "phytohormones" of the auxin and heteroauxin groups. The hypertrophy of the coleoptile, the pseudoneoplasias of the roottips and hypocotyl obtained in wheat seedling, and accompanied by inhibition of straight growth, the stimulation of the somatic growth of Begonia, the local initiation of roots on the stem of Impatiens, are a few of the examples reported in previous communications (8, 9, 14, 15, 16). Some analogous effects of colchicine have since been confirmed by A. F. Blakeslee (2), P. and N. Gavaudan (13), and O. Eigsti (11).

I have further tested the supposed "hormonal" action of colchicine by the following methods:

1. Radish seeds were germinated in Petri dishes on filter paper moistened with a 1/1000 aqueous solution of colchicine. Immediately after the appearance of the cotyledons the young plants were transferred in pots filled with garden soil, no more colchicine being added when watering. As a result of this treatment the "tuberization" of the roots in the form of small radishes, had begun approximately at the same time as the unfolding of the cotyledons, whereas the control plants treated with water only have shown no signs of tuberization even 12 days after, when already 3 pairs of leaves had been produced.

2. The dorsal or ventral surface of the leaves of young tomato plants was gently rubbed with a glass spatula or the finger tip dipped in a 5/1000 aqueous solution of colchicine. The leaves treated on their upper surface showed within 30–40 minutes a very marked epinastic response, which lasted from 4 to 6 hours. Although distinct enough, the hyponastic effect of the treatment of the *underside* of the leaf surface was less marked and did not last as long. Plants similarly treated with phenylurethane gave no nastic responses whatever, although, as I have experienced, this substance produces in wheat seedlings almost exactly the same phenomena as colchicine.

3. Tradescantia (var. Zebrina pendula) sprayed with a 1/2000 solution of

colchicine displayed, in spite of its long trailing stems, in a surprisingly short time (15 to 25 minutes) a marked hyponastic response. Prolonged treatment (6 weeks) repeated every third day caused a gradually increasing retardation of the hyponastic effect and the diminution of its intensity. On the other hand while the elongation of shoots and the development of new stems had been inhibited, the initiation of adventitious roots on the stems was distinctly stimulated. As it can be seen, all the above effects of colchicine are in marked extent in accordance with those obtained amongst others by Fischnich (12), Laibach (20), Lefèvre (21), Solacolu (26), Borthwick (4) and the workers of the Boyce Thompson Institute (19, 28, 29) when treating plants with heteroauxin or other "growth substances" of this group.

4. Cherry-laurel leafs wounded by removing small disks of leaf tissue with a cork borer produce as G. Samuel (24) has described the Clasterosporium or "shot hole" effect. This consists in the formation at a certain distance from the wound of one or more concentric abscissa lines of incompletely suberized cells and the subsequent dropping out of the rings of leaf tissue situated between the abscissa lines and the wound. I have found that in a given time the rate of production of the abscissa lines and their distance from the wound (and in consequence also the area of leaf tissue discarded) is in inverse relation to the time required for the healing of the wound when treated with different substances according to their ability to stimulate (Hamamelis) or prevent (Viscum) the healing of wounds. When wounds produced as mentioned were painted with colchicine (1/2000 to 2/1000) their healing was obtained within a few hours and no abscissa line was formed in the same laps of time in which the control wounds (treated with water) had already produced two, involving the discarding of one or two rings of leaf tissue. Thus, while the diameter of the wounds treated with colchicine remained restricted to that of the disk of leaf tissue originally removed, the diameter of wounds treated with water only, increased by 50 to 100 percent. In this instance colchicine showed in consequence a certain analogy of action with Haberlandt's (13a) "wound hormone" or the "traumatin" of Bonner and English (3).

5. On the other hand, the stimulation of yeast growth by colchicine as reported by Richards (23) and that of Penicillus, as I have accidentally noticed myself, point to a Bios or Vitamin B-like action (Schopfer (25)) of this substance.

6. It remained to show by one of the more specific tests how far, and in what sense, a claim to inscribe colchicine on the list of growth promoting substances or "phytohormones" is justified. The classical "pea test method" of Went gave however in all the otherwise active concentrations entirely *negative* results.

In view of all these Protean and somewhat conflicting manifestations of the action of colchicine I hesitate to consider this substance as either specific or as a true plant hormone.

On the other hand the above outlined experimental evidence appears to confirm the theory previously exposed (4) i.e. that the "hormonal" action of colchicine is exercised through: 1° the mobilisation of the hormones already present

in the plants; 2° the deviation of the polarity of their transport, causing a more or less localised "hyper-" or "hypo-auxonomy"; 3° the localised or general inactivation of the endogenous phytohormones ("auto-auxones").

The preliminary experimental evidence which tends to prove that such an action is actually exercised by colchicine on *at least* one of the phytohormons (Vitamin C) is the following: a) the Vitamin C (ascorbic acid) content (kindly estimated by Dr. E. Gàl) of wheat seedlings and of young tomato plants is reduced when treated with colchicine; the above mentioned atypical growth (pseudoneoplasias) of wheat seedlings and the epinastic response of tomato plants is inhibited when Vitamin C is added to the solutions of colchicine. The circumstance that ascorbic acid stimulates the development of "phytocarcinomata" (crown gall) (10) and that colchicine inhibits their growth (4) adds interest to these observations.

Although it is by no means suggested that the above preliminary experiments and the deductions drawn therefrom suffice to establish a specific action of colchicine on any singular plant hormone some of the manyfold and apparently paradoxical effects of this alkaloid can find thus a plausible explanation. Moreover the suggestive parallelisms which can be established between the above and other reactions of plants and those obtained by A. P. Dustin and his school (6, 7, 8, 9) in animals treated with colchicine seem to assign to this substance an important rôle as a revealer of some of the secrets of the mechanism of normal and atypical growth.

Brussels, 28 May 1938.

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# TEMPERATURE AND RELATIVE GROWTH OF CHICK EMBRYO LEG BONES

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The allometric equation of Huxley $y = bx^{\alpha}$ has been found to describe adequately the average trends in the growth pattern of the leg bones of the chick. Although as Lerner (1938a) has shown for post-hatching growth there exist variations in the $\alpha$ values of individuals with respect to age, it is possible to obtain valid least squares determinations of the allometric constants both for chick embryos (Lerner, 1936) and for the post-hatching period of growth (Lerner, 1937). What the significance of these constants is, presents a debatable question. Thus Richards (1935) considers them to be an unjustified over-simplification which tends to obscure the true nature of growth. On the other hand, it has been repeatedly stated that, although the relative growth function may be an approximation, it is still a useful one in describing certain trends of dimensional relationships (Huxley 1932, Lumer 1937). It may be true that its indiscriminate use may lead to erroneous interpretations. At the same time, it doubtlessly expresses the average ratio between the growth rate of one part and of another or of the whole. While the apparent constancy of $\alpha$ values over a period of time may be illusory, it is more than a coincidence that similar if not identical values are obtained for the same structures in different breeds of fowl (Lerner, 1937), of horses (Walton and Hammond, 1938) and of cattle (Pontecorvo, 1938), or still more strikingly for the same chemical constituents of the organism in different species (Needham, 1934). An apparent chemical ground-plan of animal growth is strongly in evidence, as is also a uniform morphogenetic plan within the species of Gallus gallus. The Bantam members of this species, however, do not appear to fall within this plan (Lerner, 1937) although investigations on them in this laboratory are not by any means complete.

It is of interest to determine, whether or not it is possible to produce deviations from such a morphogenetic plan by varying the growing conditions of an animal. Such differences have been found by Lerner (1938a and b) in birds hatched at different times, but no relation between the rate of growth and changes in the growth ratio was observed by him. On the other hand, Outhouse and Mendel (1933) found that appendicular bones in rats with a slow rate of growth were always longer at the same body weight than those of rats growing rapidly. It would seem thus, that there may be a difference in the allometric coefficients between the rats grown under different conditions. However, examination of the data in their Table 5 leads to the conclusion that the difference lies in the value of the constant $b$, while $\alpha$ is the same or slightly higher in the rapidly growing rats. This means, that whatever advantage the slowly growing animals have in the length of bones has accrued before the two groups have reached the weight of 60 grams, which is the earliest recorded. The $\alpha$ value be-

yond that weight is either the same in the two groups or tends to show a compensatory increase in the rapidly growing group.

Moment (1933) pursuing his investigations on the same group of animals found a general dependence of the size of parts on body size, rather than on age, which finding suggests an allometric relationship. The growth of the eyeballs

TABLE 1

MEAN AND STANDARD ERRORS OF WET EMBRYO WEIGHTS (IN GRAMS) AND BONE LENGTHS (IN CENTIMETERS)

| Days | 98° | | | |
|---|---|---|---|---|
| | Weight | Femur | Tibiotarsus | Tarsometatarsus |
| 10 | 1.13± .051 | .34± .015 | .47± .021 | .21± .014 |
| 11 | 1.63± .065 | .48± .020 | .64± .019 | .36± .014 |
| 12 | 2.13± .085 | .61± .021 | .75± .028 | .47± .026 |
| 13 | 2.59± .099 | .63± .019 | .77± .024 | .51± .005 |
| 14 | 3.54± .136 | .76± .022 | .97± .023 | .70± .022 |
| 15 | 4.76± .186 | .91± .025 | 1.19± .028 | .78± .032 |
| 16 | 6.78± .189 | 1.11± .023 | 1.44± .025 | 1.00± .036 |
| 17 | 8.81± .445 | 1.24± .038 | 1.59± .035 | 1.14± .035 |
| 18 | 10.85± .490 | 1.38± .051 | 1.84± .039 | 1.37± .034 |
| 19 | 15.11± .873 | 1.52± .041 | 2.02± .086 | 1.58± .041 |
| | 101° | | | |
| 10 | 1.59± .062 | .42± .015 | .53± .023 | .31± .021 |
| 11 | 2.16± .086 | .60± .022 | .81± .031 | .48± .022 |
| 12 | 2.81± .120 | .68± .017 | .93± .024 | .56± .028 |
| 13 | 4.06± .107 | .77± .013 | 1.05± .017 | .74± .017 |
| 14 | 5.60± .234 | .85± .023 | 1.19± .036 | .76± .023 |
| 15 | 7.69± .295 | 1.10± .037 | 1.59± .034 | 1.00± .033 |
| 16 | 10.00± .305 | 1.19± .022 | 1.65± .040 | 1.15± .022 |
| 17 | 13.06± .085 | 1.41± .035 | 1.93± .036 | 1.42± .034 |
| 18 | 15.38± .241 | 1.62± .023 | 2.24± .040 | 1.68± .025 |
| 19 | 19.60± .781 | 1.76± .037 | 2.37± .037 | 1.87± .031 |
| | 104° | | | |
| 10 | 2.54± .060 | .64± .034 | .81± .042 | .51± .035 |
| 11 | 3.79± .130 | .66± .036 | 1.07± .034 | .60± .024 |
| 12 | 5.46± .091 | .94± .021 | 1.25± .021 | .83± .019 |
| 13 | 7.95± .221 | 1.09± .038 | 1.54± .039 | 1.01± .028 |
| 14 | 10.66± .346 | 1.22± .021 | 1.77± .032 | 1.14± .030 |
| 15 | 13.86± .401 | 1.42± .033 | 2.05± .041 | 1.38± .042 |
| 16 | 17.88± .516 | 1.71± .029 | 2.34± .028 | 1.74± .040 |
| 17 | 20.66± .428 | 1.75± .031 | 2.54± .042 | 1.82± .034 |
| 18 | 24.04± .430 | 1.94± .029 | 2.82± .046 | 2.06± .048 |
| 19 | 27.20± .446 | 2.07± .026 | 2.97± .048 | 2.19± .038 |

on the other hand depended on the age of the animals and as Richards (1935) was later able to show exhibited a varying growth ratio.

An analogous experiment with chick embryo leg bones was undertaken. Since the rate of embryo growth depends on the incubation temperature it was possible to control it by differential temperatures. Accordingly three groups of eggs were set in adjoining compartments of an electrified natural-draft, sectional incubator, regulated to temperature readings of 98°, 101° and 104° F. respectively with the center of the thermometer bulb located about 5 cm. above the bottom of the egg tray. The temperature varied during the course of incubation

only within very narrow limits. The growth observed in the 104° group was approximately equivalent to that obtained in embryos incubated at 99.5° F. in a forced-draft, cabinet type incubator.

The eggs set originated from the Experiment Station flock of Single Comb White Leghorns and were selected from four days' production previous to the day of setting, each compartment having the same number of eggs of the different sizes used. Thus each compartment contained 38 eggs in each of four two-gram egg weight classes from 55.0 to 62.9 grams. In passing, it may be mentioned that egg weight was found to have no effect on embryo growth within the incubation period and range of egg weights studied.

Starting at 10 days of incubation and daily until the 19th day, 10 eggs containing living embryos as determined by candling were removed from each compartment and immersed in ice water immediately to arrest development. Infertility and embryonic mortality reduced the number of embryos in the 98° group, so that only five were available for the 19th day. The eggs were broken out and after removal of the amniotic membranes the embryos were rolled on paper towels and weighed to the nearest milligram. They were then tagged for identification and placed in a 5 percent solution of formalin to be preserved for measurement. Two to five days after each weighing the leg bones were measured with pointed calipers, the readings being taken off a rule marked in half millimeters. Exact measurements were taken on the femur and the tibiotarsus, while the tarsometatarsus measurement extended at the distal end to the center of the ball of the foot.

TABLE 2

ALLOMETRIC CONSTANTS BASED ON SIX VALUES IN A SIMILAR EMBRYO WEIGHT RANGE FOR EACH TEMPERATURE GROUP

| Temperature group | Constant | Femur | Tibiotarsus | Tarsometatarsus |
|---|---|---|---|---|
| 98° | Growth ratio | .51± .199 | .59± .057 | .65± .051 |
| | log $b$ | .60 | .66 | .45 |
| 101° | Growth ratio | .48± .076 | .50± .079 | .58± .099 |
| | log $b$ | .60 | .73 | .49 |
| 104° | Growth ratio | .50± .098 | .54± .030 | .60± .066 |
| | log $b$ | .58 | .70 | .46 |

Mean wet embryo weights and measurements with the respective standard errors are given in Table 1. It may be noted that the 104° group was approximately two days ahead of the 101° group and three days ahead of the 98° group. Determination of the allometric constants for the three groups was confined to the six ages per group which had a common embryo weight range. Thus the 98° group included the ages from 13 to 18 days, the 101° group—from 12 to 17 days and the 104° group—from 10 to 15 days. The 19th day measurements of the first group were not used because of the reduced number of embryos available for this stage.

Table 2 presents the values of $\alpha$ and log $b$ for each of the three leg bones for

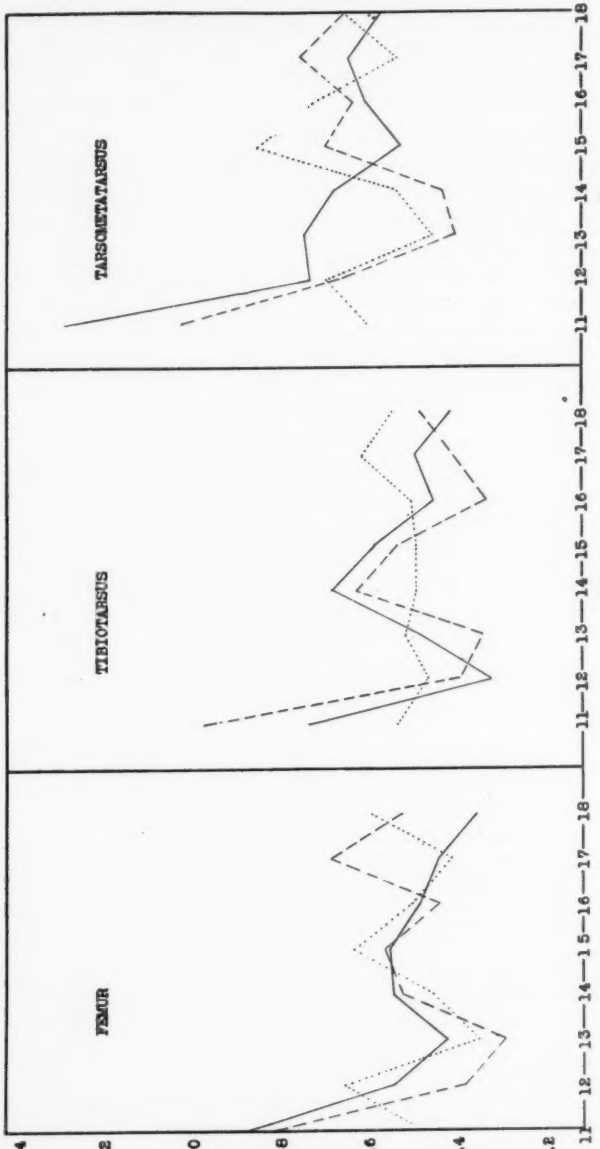


FIG. 1. Instantaneous growth ratios. Solid line—98° group; broken line—101° group; dotted line—104° group.

the three groups. It is apparent that the three groups do not differ from each other with respect to the growth ratios observed. The distal proximal growth gradient previously observed in Landauer's material (Lerner, 1936) is once more seen. It does not have a uniform slope in the three groups, but the variations do not appear to be regular.

The data here presented thus do not indicate any departure from the postulated uniform growth pattern as determined from average measurements (see Hersh, 1938). However, the possibility of significant deviations from the average trend is not excluded.

A technique such as used by Richards (1935) in examining the data of Moment (1933) was used for a study of this point. Percentage rates of growth (differences between natural logarithms of successive weights or measurements) were computed for embryo weight and for the bone measurements and the ratio of the latter over the former considered as the *instantaneous* $\alpha$ value at each particular growth stage.

The percentage growth rates of all weights and measurements, were smoothed by the shifting average method and $\alpha$ values obtained from the corrected rates. Figure 1 presents the fluctuations of the growth ratio for each of the three bones. Although, as in the case of Moment's data (see Figure 5 of Richards) the variation observed suggests that the $\alpha$ values in Table 2 are only an expression of a general trend, no regularities in the fluctuation can be observed either with age or temperature. The pattern of the 98° group seems closer to that of the 101° group than either of the two are to that of the 104° group, the latter showing greater uniformity, but beyond this statement not much more can be said. Using the logarithms of age for the abscissae in Figure 1 does not aid in the interpretation of the curves obtained.

The question of true ages of the three groups studied is an important one. The chronological age is not comparable in the three groups of embryos. Having reached similar weights after different durations of growth, the embryos may be of the same morphological age. Apparently, so far as the proportions of the leg bones with regard to embryo weight are concerned this is true. It may then be concluded that the experiment reported here tends to support the view that the general pattern of growth of the leg bones, as expressed by *average* allometric growth ratios depends entirely on embryo weight, although *instantaneous* growth ratios show variations, which are unrelated to the weight increase of the embryo.

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# MONTHLY WEIGHT CHANGES OF WHITE LEGHORN PULLETS IN RELATION TO CONCURRENT EGG WEIGHT AND RATIONS

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## INTRODUCTION

One of the well recognized objectives of commercial egg producers is to hasten the attainment of sexual maturity of pullets as much as possible without producing a supposedly detrimental effect on body size or egg weight. As a general rule the efforts to obtain rapid growth of pullets are restricted largely because of the popular opinion that attempts to speed the growth process stimulate the production of eggs before the pullets have attained a sufficient proportion of their ultimate body size. There is even the extreme opinion that pullets may never attain the size of which they are genetically capable if they are "forced" into production too early in life.

The factors which constitute the forcing process are not entirely known but the feeding of rations rich in proteins is traditionally assumed to be one of the major factors. Another factor is undoubtedly the influence of the amount or wave length of light for Bissonnette (1933), Dakan (1934) and others have shown that light stimulates the functioning of the reproductive processes. The influence of a long light day at a time when pullets are approaching body maturity may offer a partial explanation of the findings of Upp and Thompson (1927) that pullets hatched December 21 tended to mature sexually in a shorter period of time than those hatched later in the winter. They also show "an intimate relationship ($.7891 \pm .0153$) between early sexual maturity and small size of the first eggs laid" and cite results of a number of other investigators in agreement. More recently Waters (1937) made similar observations with respect to the relationship of egg size to age at maturity. If it is possible to hasten sexual maturity by the use of any factor, especially a ration, it seems reasonable to expect that such factors would be associated with small eggs because of a general correlation between body weight and egg weight. A possible exception might be a ration which contains materials which are especially suitable for the development of the body frame work of the fowl.

Evidence that mean annual egg weight of pullets appeared to be negatively associated with the percentages of protein in the rations was presented by Henderson (1937). At that time it was suggested that while the evidence seemed inconclusive this unexpected negative regression of egg weight on the protein content of the rations might be the result, in part, of the kind of proteins which

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were fed. It seemed possible that certain protein supplements might stimulate a rate of egg production out of proportion to the rate of body growth, but it was not supposed that any of the rations were deficient in materials for growth, especially calcium and phosphorus.

There is a rather widespread belief among poultrymen that large body frame work is essential to the production of large eggs but there are very few reports of systematic studies of the association of these two factors. Asmundson (1921) seems to be one of the first if not the only investigator who has reported correlations between several body measurements of the hens and the weight of the eggs laid. The criterion which is most highly correlated with egg weight in Asmundson's report is body weight ($.3838 \pm .0344$). The lowest body measurement correlation he found was that between the depth (keel to pelvis) $.2194 \pm .0383$. Other correlations were: "Total egg production—$.0118 \pm .0403$."

"Dist. back—Posterior end of keel. $.3593 \pm .0351$.

"Distance between pelvics. $.2438 \pm .0379$.

"Weight of bird and length of keel. $.3146 \pm .0363$.

"Weight of bird and width at hips. $.6851 \pm .0214$."

A number of other investigators have studied the relationships between average body weight and average egg weight with various objectives in mind and as a result the methods of calculating averages and results differ somewhat. To date all reports seem to agree that mean body weight at various ages and average egg weight as calculated by various methods are correlated. Funk (1935) has called attention to a number of studies of the above nature and it seems unnecessary to review all of them at this time.

Even though it has been known for many years that there is a significant positive correlation between body weight of hens and the weight of eggs they lay, the problem of increasing the number of large eggs still exists. To make a literal and widespread application of the knowledge of correlations would mean that commercial poultrymen should be keeping large hens such as Brahmas and Cochins. Limitations to increasing egg size by selecting large breeds are suggested in a report by Marble (1930) which demonstrates that the regression of mean egg weight on mean annual body weight of pullets is not linear and that the value of the regressions decreases as the mean weight becomes greater. With hens he found that the regression is linear. He suggests that "it is obviously undesirable to increase the size of the bird to the point where the slightly greater size obtained with respect to egg weight is not entirely overcome by the greater cost of maintenance as the result of supplying nourishment for the larger body."

The problem of getting large sized eggs from pullets which are growing and laying at the same time is more serious than it is with hens. It is well known that pullets of practically all the common breeds lay smaller eggs when they begin production than they lay after their first six months of production. Very few reports have been published in which the relationship of the rate of growth of the pullets and the rate of increase of egg weight are compared. Atwood (1923) made a comparison of pullet growth and increase in egg weight and found a "reasonably regular increase in the weight of the pullet eggs from the beginning

of the laying year. He stated that with pullets "the mean weight of the eggs increased concurrently with the increase in the weight of the birds."

Later Atwood and Clark (1930) compared the weights of eggs and body weight of fowls during the first three years of production. The rations fed were a combination of grain and mash and were the same for all birds. They found the following correlations:

Egg weight 1st year and body weight that year. .458 ± .040.
Egg weight 2nd year and body weight that year. .434 ± .040.
Egg weight 3rd year and body weight that year. .426 ± 043.

Although annual mean body weight is correlated for all three years with annual mean egg weight in Atwood and Clark's report there may be some question as to the biological significance or utility of a correlation calculated in this manner. Since pullet weight and egg weight are increasing at least during the early part of the year the means as determined by series of consecutive observations may have an *arbitrary value.*

Byerly, Titus and Ellis (1933) use body weight at the end of the pullet year as an index of pullet weight and correlate it with "average egg weight." It is not clear in their paper how average individual egg weight was determined. Final body weight is perhaps a more useful index for some purposes than average body weight, but if the influence of a ration is measured in relation to egg weight over the period of a year, it is probably worth while to know what is happening concurrently to body weight. How to measure the degree of concurrence or parallelism of egg weight and pullet weight is a somewhat tedious mathematical problem. The determination of correlation coefficients between such indices as annual mean egg weight and pullet weight is probably too irrelevant but the information which might be obtained by more precise mathematical procedure may not be worth the expense. For some purposes a visual comparison of the curves of egg weight and pullet weight as plotted for these values on graphs may be satisfactory.

From the graphs in Atwood and Clark's report of 1930 there is some evidence that body weight does not limit egg weight because the curve of mean egg weight increased to a peak in December of the first year while mean hen weight declined for the same period. A similar marked divergence is apparent during the period from October to January of the second year. This suggests that the capacity of the body of the bird rather than body weight may be the limiting factor on egg size although body weight is commonly taken as the most convenient substitute for capacity measurements. This probable distinction should be kept in mind for it has become a rather common practice in recent years to recommend the feeding of a "fattening" mash to pullets in the fall on the popular supposition that pullets stop laying and go into a molt as the result of losses in body weight. If the development of a large body framework is the limiting factor on egg size, pullets should be given a ration in the autumn with a narrow nutritive ratio and a high calcium and phosphorus content rather than a fattening mash.

The determination of correlation coefficients between broad criteria such as annual mean body weight and annual egg weight is no doubt of value as an indication of what egg size is associated with but the utility of such information is of limited value without consideration of the periodic changes which affect the means. It is well recognized, of course, that the method of correlation is commonly the only means of measuring the degree of association of two variables but it is also recognized that two variables may be correlated without either being biologically dependent on the other. For example, it is conceivable that while body weight of growing pullets and egg weight may be correlated normally, nevertheless it may be impossible to affect egg weight by the experimental alteration of body weight because of the possibility that egg weight may depend more on size of body frame work which, presumably cannot be altered once it is established. If egg size is dependent on some criteria such as the interior body capacity or the length or volume of the oviduct, the practice of increasing body weight by increasing the amount of internal fat may be detrimental to the rapid attainment of maximum egg weight.

If correlation coefficients are calculated between two sets of values such as egg weight of pullets in successive months and body weight in successive months during the growing period, positive values might be expected for both values are normally increasing during this period. A part of the increase in body weight may be attributed to the accumulation of ova in the ovary.

Knowledge of how egg weight may be affected experimentally should be the ultimate goal of research workers interested in this problem.

## EXPERIMENTAL PROCEDURE

### Object

The purpose of the study reported herewith was to determine whether or not there are differences in correlations between the rate of change in body weight of pullets and the rate of change in egg weight during the growing period which may be attributed to the quantity or quality of protein supplements in the rations and whether or not rations which contained the larger proportion of muscle and bone building materials, i.e., those which contained a high percentage of protein, calcium and phosphorus, had an accelerating influence on the attainment of maximum egg weight.

### Method

The method of selection and management of the eight pens of pullets are described in a previous report (Henderson, 1937).

The data recorded consist of the following: (1) The monthly body weight of 192 single comb White Leghorn pullets which lived throughout a 12 month period. (2) The weight of each egg laid daily by pullets in nests. (3) The average weight of a known "all mash" mixture of feed consumed per pullet per month during the assumed "growing" period, October—February.

The group of 192 pullets was divided into 8 pens each of which was fed the ration indicated in Table I. The percentage of protein in the mixed rations was

TABLE I
RATIONS

| Basal Ration | Pen Numbers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | Ingredients—Pounds | | | | | | | |
| Wheat | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Oats | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| Corn | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| Bone meal | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Salt | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Cod liver oil | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Total | 105 | 105 | 105 | 105 | 105 | 105 | 105 | 105 |
| Supplements | | | | | | | | |
| Milk | 15 | 10 | 5 | | 5 | 9 | 5 | |
| Meat and bone meal | | | | 10 | 5 | 5 | 8 | 5 |
| Totals | 120 | 115 | 110 | 115 | 115 | 119 | 118 | 110 |
| Percent Protein | 14.40 | 13.41 | 12.32 | 14.52 | 13.96 | 14.75 | 14.88 | 12.90 |

estimated from average analyses but the percentages of protein in the concentrates were determined by chemical analyses.

The average number of pounds of feed consumed per pullet per month in each pen during the growing period is shown in Table II. Individual feed consumption records were not possible because the pullets were not fed from individual feed containers.

TABLE II
FEED CONSUMED BY PENS

| Pen No. | Average No. of pounds of feed consumed per pullet per month | | | | Average first 4 months |
|---|---|---|---|---|---|
| | Oct. | Nov. | Dec. | Jan. | |
| 1 | 5.8 | 7.1 | 5.9 | 7.0 | 6.4 |
| 2 | 5.6 | 6.9 | 5.6 | 8.4 | 6.6 |
| 3 | 6.7 | 6.3 | 6.0 | 6.4 | 6.3 |
| 4 | 5.8 | 7.2 | 5.5 | 6.8 | 6.3 |
| 5 | 5.8 | 7.1 | 5.8 | 8.6 | 6.8 |
| 6 | 6.0 | 7.0 | 5.5 | 7.6 | 6.5 |
| 7 | 5.7 | 7.1 | 4.7 | 7.5 | 6.2 |
| 8 | 5.9 | 6.2 | 5.1 | 7.3 | 6.1 |

## ANALYSIS OF DATA

In many of the reports of other studies of the relationship between egg weight and pullet weight, comparisons have been made between yearly means determined by averaging the weights taken during several successive months of the year. The means determined in this manner are obviously influenced materially by observations taken before either pullet weight or egg weight have attained mature values and to a lesser extent after these values are attained. Such means are arbitrary and lack utility for certain comparative purposes since they represent the weight of pullets at unspecified instants of time.

In this study an attempt has been made to distinguish between correlations

which may exist during the growing period when pullets are still growing and laying eggs which are successively heavier, and correlations during the period when mature body weight or size and egg weight are approximately established.

## Relationships During the Growth Period of Pullets

The period during which body weight and egg weight appeared to be increasing at nearly constant rates was selected for the initial comparisons since the graphs of these functions provided curves which could be easily converted to linear forms.

Table III

Mean Egg Weight and Mean Pullet Weight per Month in Pens I to VIII

| Pen No. | I | | II | | III | | IV | |
|---|---|---|---|---|---|---|---|---|
| | Egg Weight Grams | Pullet Weight Pounds | Egg Weight | Pullet Weight | Egg Weight | Pullet Weight | Egg Weight | Pullet Weight |
| October | 42.8 | 3.23 | 44.2 | 3.21 | 43.7 | 3.15 | 41.7 | 3.16 |
| November | 45.5 | 3.40 | 46.6 | 3.40 | 45.9 | 3.39 | 44.0 | 2.92 |
| December | 44.2 | 3.26 | 47.1 | 3.49 | 47.9 | 3.56 | 44.8 | 3.13 |
| January | 48.9 | 3.49 | 49.5 | 3.59 | 50.0 | 3.64 | 47.6 | 3.49 |
| February | 49.6 | 3.98 | 49.0 | 3.90 | 49.8 | 3.86 | 49.1 | 3.55 |
| March | 50.1 | 3.94 | 51.3 | 4.02 | 52.3 | 3.95 | 50.6 | 3.91 |
| April | 50.4 | 4.23 | 50.7 | 3.97 | 52.6 | 3.89 | 50.5 | 3.75 |
| May | 50.7 | 4.06 | 50.5 | 3.85 | 52.9 | 3.89 | 50.9 | 3.87 |
| June | 50.3 | 3.98 | 49.3 | 3.78 | 51.6 | 3.86 | 49.5 | 3.68 |
| July | 49.1 | 4.01 | 49.1 | 3.62 | 48.7 | 3.60 | 48.9 | 3.47 |
| August | 50.8 | 3.72 | 50.5 | 3.46 | 52.1 | 3.41 | 50.2 | 3.56 |
| September | 50.6 | 4.05 | 52.2 | 3.84 | 53.2 | 3.78 | 52.2 | 3.91 |

| Pen No. | V | | VI | | VII | | VIII | |
|---|---|---|---|---|---|---|---|---|
| | Egg Weight | Pullet Weight | Egg Weight | Pullet Weight | Egg Weight | Pullet Weight | Egg Weight | Pullet Weight |
| October | 43.5 | 3.06 | 43.8 | 2.97 | 43.0 | 3.04 | 42.5 | 3.32 |
| November | 46.1 | 3.19 | 46.4 | 3.17 | 45.0 | 3.23 | 44.7 | 3.27 |
| December | 47.5 | 3.39 | 46.9 | 3.46 | 46.3 | 3.47 | 46.7 | 3.11 |
| January | 50.5 | 3.49 | 50.6 | 3.57 | 49.6 | 3.64 | 48.7 | 3.35 |
| Feburary | 51.5 | 4.02 | 51.4 | 3.99 | 49.7 | 3.96 | 49.5 | 3.59 |
| March | 52.2 | 4.17 | 51.4 | 4.07 | 51.4 | 4.02 | 51.7 | 3.62 |
| April | 52.2 | 4.06 | 52.2 | 3.93 | 51.3 | 3.93 | 51.9 | 3.78 |
| May | 51.9 | 4.04 | 51.8 | 3.88 | 50.9 | 3.94 | 51.9 | 3.84 |
| June | 50.2 | 3.98 | 50.7 | 3.96 | 49.1 | 3.79 | 50.8 | 3.87 |
| July | 49.8 | 3.61 | 49.7 | 3.58 | 49.1 | 3.39 | 50.3 | 3.42 |
| August | 51.2 | 3.63 | 49.8 | 3.49 | 49.9 | 3.51 | 52.1 | 3.48 |
| September | 54.7 | 3.90 | 53.6 | 3.72 | 50.5 | 3.63 | 52.7 | 3.72 |

The mean pullet weight per month and mean egg weight per month are shown in Table III. The data are plotted on semilogarithmic graphs in order to visualize rates of change in weights by months and for comparative purposes. Rates of change of egg weight and pullet weight were calculated for all the individual birds for the period October to January inclusive by Brody's (1927) method. This period was arbitrarily chosen as the most critical "growth" period for comparison but it is obvious from Figure 1 (Pens 1–8) that increases in both pullet weight and egg weight continued beyond the month of January in most of the

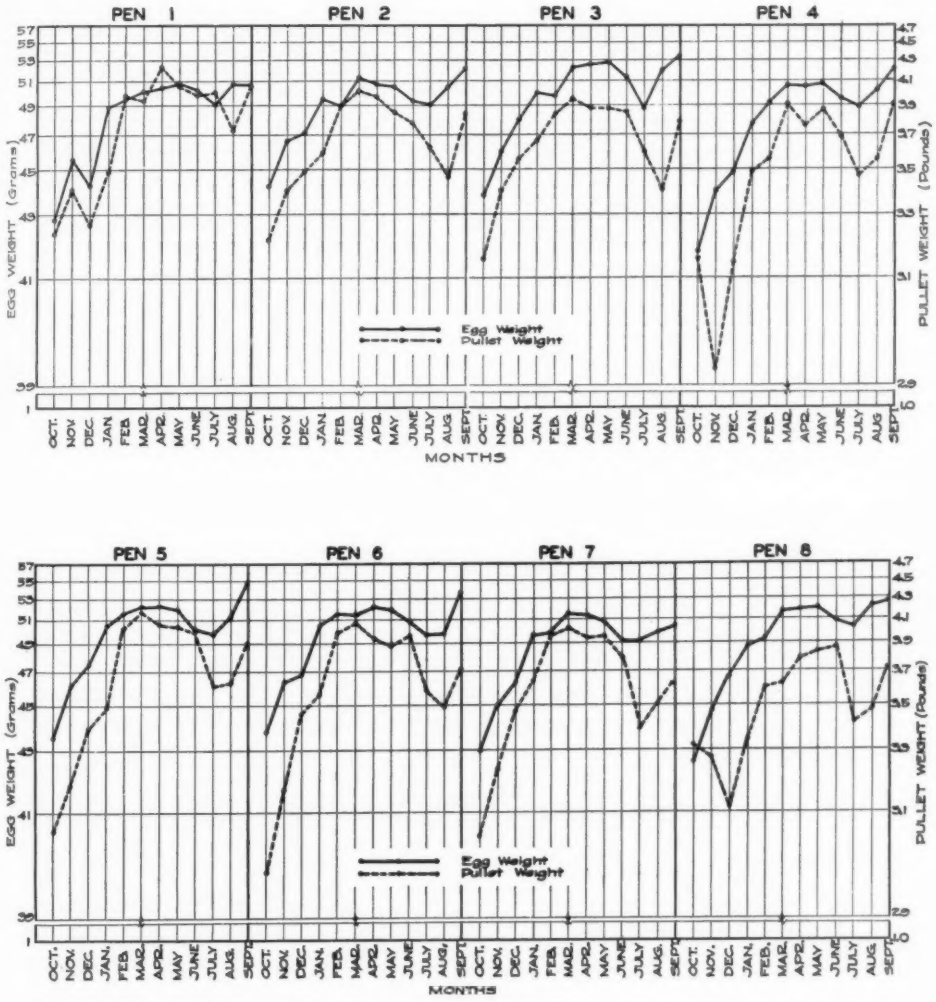


FIG. 1. Monthly weight changes of white leghorn pullets in relation to concurrent egg weight and rations.

pens. The mean rates of increase of pullet weight and egg weight are indicated in Table IV.

In spite of the apparent difference in rate of increase of mean pullet weight between some of the pens general significance was not indicated (for the mean

TABLE IV

MEAN PERCENTAGE RATES OF INCREASE OF PULLET WEIGHT AND EGG WEIGHT From October to January

| Pen No. | Number of Pullets | Mean Percentage Rate of Increase of Pullet Weight | Mean Percentage Rate of Increase of Egg Weight |
|---|---|---|---|
| 1 | 24 | 2.40 | 1.65 |
| 2 | 23 | 2.11 | 1.40 |
| 3 | 25 | 1.70 | 2.11 |
| Group 1 | (72) | 2.07 | 1.73 |
| 4 | 21 | 1.77 | 2.40 |
| Group 2 | (48) | 1.46 | 2.04 |
| 5 | 23 | 1.99 | 2.00 |
| 6 | 27 | 2.31 | 1.60 |
| 7 | 22 | 2.66 | 1.37 |
| Group 3 | (72) | 2.31 | 1.87 |
| 8 | 27 | 1.22 | 1.76 |
| Totals | 192 | 2.00 | 1.86 |

Group 1 = Pens 1, 2, 3. Group 2 = Pens 4 and 8. Group 3 = Pens 5, 6, and 7.

square of the variation within pens was greater than the mean square between pens). Lack of general significance between pens was also determined for rate of increase in egg weight.

## CORRELATIONS BETWEEN RATES OF INCREASE

One of the principle objects of this study was to determine whether or not body weight influences egg weight and whether the rations might affect such a relationship. If body weight influences egg weight it might be expected that rates of increase of these two functions would be similar and that the extent of such similarity might be expressed by means of correlation coefficients.

TABLE V

CORRELATION COEFFICIENTS BETWEEN RATE OF INCREASE IN BODY WEIGHT AND RATE OF INCREASE OF EGG WEIGHT OF SINGLE COMB WHITE LEGHORN PULLETS

| Pen Numbers | Degrees of Freedom | Correlation Coefficient |
|---|---|---|
| Between pens | 6 | −.1994 |
| 1 | 22 | .2865 |
| 2 | 21 | .2633 |
| 3 | 23 | .1461 |
| Group 1 | (71) | (.1924) |
| 4 | 19 | 0.0370 |
| Group 2 | (47) | (−.0656) |
| 5 | 21 | .2693 |
| 6 | 25 | −.2066 |
| 7 | 20 | −.0217 |
| Group 3 | (71) | (.0261) |
| 8 | 25 | −.1267 |
| Total within | 183 | .0674 |
| Total | 190 | .0587 |
| Between groups | 1 | .6585 |

The correlation coefficients between rates of increase in body weight and rates of increase of egg weight were calculated in an analysis of covariance and shown in Table V. None of the coefficients are significant for the number of degrees of freedom indicated in the table. The highest correlation is shown for Pen 1 (.28). From Wallace and Snedecor's tables a correlation of .39 is required for significance with a number as low as 22.

It may be noted from Table V that the correlations in the first three pens (which were fed dry milk) are positive and the correlations in Pens 4 and 8 (which were fed meat and bone meal) are low or negative.

In order to test for significance between pen means the analysis of covariance was extended to include the adjustment of the means of the rate of increase in egg weight for the influence of the rate of increase in pullet weight. The sum of squares of the errors of estimate of rate of increase in egg weight after the influence of pullet rate was removed are shown in Table VI. It is obvious that the difference between the mean squares is not significant.

TABLE VI

ANALYSIS OF ERRORS OF ESTIMATE OF EGG RATE CORRECTED FOR PULLET RATE

| | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 190 | 3.866 | |
| Within | 183 | 3.693 | 0.020 |
| Between pens | 7 | 0.173 | 0.025 |

## MILK COMPARED WITH MEAT AND BONE MEAL AND COMBINATIONS

In order to compare the effect of the different types of protein supplement the eight pens are arranged in three groups. Group I consisted of pens 1, 2 and 3 all of which were fed milk as a protein supplement. Group 2 consisted of Pens 4 and 8 which were fed meat and bone meal and group 3 consisted of Pens 5, 6 and 7 which were fed combinations of milk and meat and bone meal. The differences between groups are not significant. The mean square of the errors of estimate for pullet rate adjusted for egg rate are compared in Table VII.

TABLE VII

ANALYSIS OF ERRORS OF ESTIMATE. EGG RATE ADJUSTED FOR PULLET RATE

| | Degrees of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 190 | 3.8666 | |
| Within | 188 | 3.8351 | .0204 |
| Between groups | 2 | 0.0315 | .0157 |

## QUANTITY OF FEED

It seems possible that the apparent lack of influence of the different percentages of protein supplements in the rations on the correlations between rates of increase in egg weight and pullet weight, might be explained by a tendency of the individual pullets to compensate for variations in quantity of supplement by varying the amount of feed eaten. This probability cannot be verified be-

cause individual feed consumption records were not possible. Records of the average quantity of feed eaten per pullet per month in the different pens during the "growth period" as shown in Table II do not indicate any regular variation in quantity consumed with the percentages of protein in the different rations. The average amount of feed consumed for the first four months in Pen 3 which received the lowest percentage of protein is only one-tenth of a pound, which is about one percent greater than the average in Pen 7 which received the highest percentage of protein.

### Monthly Changes in Body Weight with Monthly Changes in Egg Weight March to September

Since comparisons of the rate of change of body weight with the rate of change of egg weight did not reveal significantly different adjusted means during the growing period it seemed desirable to make analogous comparisons for the balance of the year. A different procedure was necessary for studying the relationships during the period March to September because mean egg weight and mean body weight did not appear to be decreasing or increasing at constant rates during this period.

The method adopted was as follows: Increases or decreases of body weight from the first day of one month to the first day of the next were compared with increases or decreases of egg weight during a period from the 15th of each month subsequent to the date body weights were determined, to the 15th of the following month. These periods were chosen because any influence which body weight might have on the yolk at least would be registered during a period of approximately two weeks prior to the laying of the completed egg. Correlation coefficients were calculated between the change in body weight and the change in egg weight as shown in Table VIII.

Table VIII

Correlation Coefficients between Changes in Egg Weight and Pullet Weight during Periods March to September

| Periods | D/F | Between Changes of Individual Pullet Weight and Egg Weight |
|---|---|---|
| March to April | 148 | .1867* |
| April to May | 148 | .0029 |
| May to June | 138 | .0723 |
| June to July | 133 | −.1536 |
| July to August | 126 | −.0002 |
| August to Sept. | 102 | −.0911 |

* Significant.

The correlations between the change in body weight and the change in egg weight are not significant except for the period March to April. In the period June to July there is a negative correlation (−.1536) which approaches significance. For 134 degrees of freedom a correlation coefficient greater than .167 would be required for significance according to tables of Wallace and Snedecor (1931).

## Rations and Time Required to Attain "Maximum" Egg Weight

Another possible measure of the influence of rations is the time required by the pullets in the different pens to attain "maximum" average egg weight. For this purpose the term "maximum" is defined as the average weight during the month in which the first major inflection of the egg weight curve occurs. From Figure 1 it is evident that the time required to attain the maximum varies with pens. The analysis of variance in Table IX shows that the variation is not significant among pens. A comparison of the time required with percentages of protein in the rations (Table X) indicates a distinct trend of reduction in time with the first three percentages of protein, vis. 12.32, 12.90 and 13.41.

Table IX

Analysis of Variance of Time Required to Reach "Maximum" Egg Weight

| | D/F | Mean Square |
|---|---|---|
| Total | 162 | |
| Between pens | 7 | 2.1353 |
| Within | 156 | 1.3087 |

Table X

Time in Months Required to Attain "Maximum" Average Egg Weight and Percentages of Protein in the Rations

| | 3 | 8 | 2 | 5 | 1 | 4 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| Time Months | 5.32 | 5.00 | 4.47 | 4.6 | 5.05 | 4.76 | 4.47 | 4.52 |
| Percent Protein | 12.32 | 12.90 | 13.41 | 13.96 | 14.40 | 14.52 | 14.75 | 14.88 |

This trend may be due to chance for the range in percentage of protein between pens is small. It is possible, however, that when the level of protein is as much as 13.41 percent that additions beyond this amount have no effect. At any rate it seemed desirable to test the significance of this trend of reduction in time required by the first three pens to attain "maximum" egg size. The results are shown in Table XI.

Table XI

Analysis of Variance Time in Months Required to Attain Maximum Egg Weight

Pens 3, 8 and 2

| | D/F | Mean Square |
|---|---|---|
| Total | 73 | |
| Between Pens | 2 | 4.30* |
| Within pens (Error) | 71 | 1.08 |
| **Reduction in Sum of Squares Between Pens Due to Regression** | | |
| Between pens | 2 | 4.33 |
| Reduction due to regression | 1 | 8.38** |
| Deviations from regression | 1 | .27 |

* Significant.
** Highly significant.

This trend in the first three pens is highly significant.

## SUMMARY

The objectives of this study were to determine whether or not correlations between the rate of increase of pullet weight and the rate of increase of egg weight, during the "growing" period (October to January) are affected by variations in protein content of the rations.

The data include monthly body weights of 192 single comb White Leghorn pullets and the daily egg weight of the same pullets during the same periods of time.

Neither the rate of increase of pullet weight nor egg weight were affected significantly by the variations in protein content of the rations. The rate of increase of egg weight of individual pullets during the growing period was not influenced by the rate of increase of body weight during the same period. The correlations between these two variables within and between pens were not significant.

Correlations between changes in egg weight with changes in pullet weight by months during the periods March to September inclusive were not significant except that during the period of March to April a positive correlation value of .1867 was obtained. This was barely significant.

The number of months required to attain maximum egg size decreased slightly with increased percentages of protein but the trend was not consistent above a protein content of 13.4 percent. Maximum egg size was attained in an average of 5.3 months when the ration contained 12.3 percent protein and 4.4 months in two pens when the ration contained 13.4 percent and 14.8 percent protein respectively.

It is suggested that egg weight may be dependent on size or capacity of body or oviduct instead of body weight. If this is the case the most rapid attainment of maximum egg size by pullets in the autumn might be expected from the rations which stimulate rapid growth of body framework, therefore "fattening" rations may delay the attainment of maximum egg size.


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# IS THE CHEMICAL DIFFERENTIATION OF CELLS OF VALUE TO THE SURGEON?*

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For many years the surgeon and the pathologist have been interested in the actual structure of cells and their organization. Recently, however, with increase in chemical knowledge we have been attempting to correlate cell structures and function, both physical and chemical, with the underlying chemical compounds. There are points of clinical value to be obtained from a survey of the cell from its resting immature state, the spare part of Reimann, to the completely differentiated and maturely functioning one of the adult organism.

Undifferentiated cells have a more or less simple, not to say uniform composition.[1] They perhaps contain simpler chemical compounds with many and diverse open bonds. As they differentiate into different kinds of cells these compounds are reconstituted.

The basic, characteristic type of compound of living organisms is protein. The basic constituents of proteins are amino-acids. These amino-acids have the power of combining in almost limitless ratios and relations. The possibilities of diversity are incalculable. As the cells differentiate these chemical compounds rearrange themselves in the direction of becoming more and more specialized. In the muscle cell myosin is formed; in the nerve cell neuroprotein, and so on. By the protein hitching to nucleic acid are produced nucleo-proteins, and thus the constituents of the nuclei are formed with their manifold and yet directed possibilities. Finally the cells reach the stage of differentiation where they are able to do their special task. When this stage is attained each cell sets up its own peculiar chemical laboratories for the production of certain kinds of compounds and for the production of certain specialized types of work. Thus the thyroid cells laboratory produces thyroxin, the salivary cells make saliva. In addition to making its special product the cell still carries on the general functions of living, such as respiration and energy exchange.[2]

Some differentiated cells have many laboratories within a single cell. That we may visualize how it is possible for one cell to do so many things, we may call in our knowledge of colloid chemistry. We know a cell may have a number of globules within it, each of which being separated by a semi-permeable membrane may act as a center of a distinct series of chemical reactions. These react at times and the general output of a cell is eventually the by-product of the internal reaction of its numerous laboratories.[3]

As a concrete example of specialization of cells let us take the breast. At the terminal buds of each lobule there are numerous undifferentiated cells. When the ovarian hormone surges into the breast about ten days before menstruation

* Read before the Atlanta Meeting 1937—American College of Surgeons.

these cells begin to multiply.[4] Some of the daughter cells stay put as "spare parts" for the next cycle. Others proceed to differentiate into epithelial cells which organize into the lining of the ducts and acini. Then differentiation proceeds to the point of their being able to manufacture their specific product, namely, milk. That is to say, the laboratories within these cells have chemically differentiated and developed to the point where they can produce this product and are ready to go to work. The final orders to proceed with the production of milk come from the anterior pituitary gland in the form of the anterior pituitary hormone and off they go.

It is known that many of these syntheses which result in the formation of these little cellular laboratories are consecutive and some are chain reactions. That is to say that if fundamentally a cell starts with the chemical called #1 and when differentiation is complete the chemical compound is #5. This #1 chemical cannot jump to #5, but according to the chain reaction must produce #2 and then #2 must produce #3, and so on until the final differentiation product #5 is produced. Now it is known that many of these reactions are irreversible. That is to say, the trend from #1 to #5 is dominant. Therefore when a cell is on the road to differentiating it will not turn back. Therefore cell dedifferentiation probably cannot occur in a completely differentiated cell.

It is a matter of common knowledge that as differentiation advances the capacity to multiply recedes. The exact point where the chemical activities[5] for division are overshadowed by those for differentiation is of course unknown. The theoretical deductions are in harmony with this for since the chemical processes for division are common to division wherever expressed while those for differentiation are specific to the kind of cell being produced, it is clear that division can only take place where cell composition is dominantly unspecific. This then means that repair of wounds, regeneration of parts, replacement of wear and tear, hyperplasias of all kinds, and tumors both benign and malignant must come from incompletely differentiated spare parts. If undifferentiated cells contain, as we know they do, these simpler chemical unions with many open bonds, many kinds of rearrangements are possible. The potency of an undifferentiated cell is multiple.[6] Which of these it will use when it differentiates depends upon internal composition and then on what chemicals it is offered from the environment.

Cancer cells do not differentiate properly and do not organize at all. Inspection of any tumor section will attest to this. It is important to know whether this failure of development is due to internal faults or to environmental inadequacy. Since it is known, and for many years, that normal cells and cancer cells when both grow together in the same environment still exhibit each its own characteristics, the conclusion is inescapable that the cancer cell does not differentiate or organize properly because of internal faults.[5] The possibility that heredity plays a part in making certain cells potentially unstable so that they become cancer is ably discussed by C. C. Little.[7]

Cancer cells do, however, differentiate to a certain degree. Some neoplasias

are poorly differentiated and some better. Of the products of chemical differentiation which are manufactured by normal cells some are of local use only while others have profound general effects on the organism as a whole.[8] Sometimes it happens that tumor cells reach the stage of chemical development where in they, too, produce specific products. An example is the mucus produced by the cells of a rectal adenocarcinoma. But this is of local importance.

When the cells are of such type that they produce substances of general influence they may become important in symptomatology, in diagnosis, in early detection of disturbance, and in prognosis. Such cells are in the granulosa cell tumor of the ovary. These may reach a stage of chemical differentiation and produce products which cause symptoms in distant parts, such as the breast. A case in point is of interest.

A woman of sixty-three was admitted to The Lankenau Hospital because of a movable, non-tender tumor of the left breast. The tumor was excised and frozen. Dr. Reimann reported the tumor as benign from section, and that there was cellular differentiation indicative of active ovarian secretion.

But this patient had passed the menopause fifteen years previously. Obviously she had no normally functioning ovaries. Examination showed, however, that there was a mass in the region of the left ovary. This was removed two weeks after the breast operation. Both ovaries were also removed at this time. And there was a small tumor on the right one and a large on the left. Both were partly cystic. The tumors were classified as granulosa cell tumors. It was evident that the differentiation of the breast tumor cells was derived from the hormone produced by the ovarian tumor; not by the ovary itself. As conclusive evidence some of the fluid from the cystic portion of the tumors was injected into spayed mice. It produced estrus therein.[9]

Here clearly a knowledge of chemical and structural differentiation development was of real significance in the diagnosis and treatment by surgery of this patient.

Another example may be found in the not uncommon arrhenoblastoma of the ovary. This may even alter the psyche of the individual. Here chemical differentiation produces a male hormone as evidenced by flattening of the breasts, development of a beard and lowering of voice pitch. Such patients sometimes cease to care for their husbands. This means that in such a patient abdominal exploration for ovarian tumor is indicated. In contrast are the cells of Brenner's tumor of the ovary. These are histologically similar to those of the arrhenoblastoma, but they differ therefrom in the very important particular that they do not chemically differentiate to the stage where the male hormone is produced.

Now since the cells of certain cancers produce recognizable and detectable products as a result of their specific inadequacy of chemical differentiation; it may well be that cells of other cancers also produce specific products. These we may in time learn to detect. Because of these facts it is clear that knowledge of chemical differentiation is of importance to the surgeon.

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# BOOK REVIEWS

THE PSYCHOLOGY OF EARLY GROWTH. Arnold Gesell and Helen Thompson. Yale Clinic of Child Development. Macmillan Co. N.Y. 290 pp. 30 Figs. 25 Tables. Index.

The volume represents continuation and elaboration of earlier work published in *The Mental Growth of the Preschool Child*. It is based on 10 years of subsequent collaborative research; part of which has appeared in *An Atlas of Infant Behavior* and *Infant Behavior; Its Genesis and Growth*. The research is based on the belief that the growth processes which mold the body and behavior of the human infant are essentially comparable with those which are being analyzed by experimental embryology. The present approach is chiefly biometric and offers useful though unique methods of statistical analysis. Part I deals with methods and procedures; Part II with the norms of infant growth; and Part III with an analytical appraisal of the growth status. Six appendices deal with comparative evaluations; bibliography of the Yale collection of films; procedure for examinations; record forms; normative summaries; and a glossary. The genetic aspect is stressed because—quoting freely—behavior growth is a changing complex which tends to follow an ontogenetic sequence characteristic of the species. Levels of maturity are manifested in analyzable patterns of behavior. Behavior growth is not a process of augmentation nor is it merely a process of progressive differentiation. It evidences itself in behavior items showing focal, decreasing, and fluctuating trends, as well as items showing increasing trends. The authors give a definition of growth which is noteworthy for its concise inclusiveness, viz. *growth is the progress of an organism toward a mature state.*

F. S. HAMMETT

PHYTOHORMONES. F. W. Went and Kenneth V. Thimann. Experimental Biology Monographs. The Macmillan Company.

This volume gives a well organized and concise review of the history, present status and probable outlook of the field of what has come to be called the "phytohormone" problem. It has been brought up to the minute and is certainly a valuable supplement to Boysen-Jensen's excellent volume "Die Wuchstofftheorie."

The present reviewer cannot help but feel, however, that it is a great pity that Went and Thimann, lacking the humility with which Boysen-Jensen presented the subject as "theorie," have chosen to present as fact, much that to any unbiased reader must seem based on very shaky evidence. The reader is repeatedly struck by this tendency to build a theory on what is stated to be fact but which on closer examination proves to be itself theory. To take an example from a field with which this reviewer is intimately acquainted: In discussing the implications of the *in vitro* cultivation of excised roots by Fiedler, White, Robbins and others the authors state that the results "certainly indicate that auxin plays no part in their growth" but add "The fact that in these cultures roots continue to branch, however, would indicate that they produce traces of auxin or else that the small amount of auxin in the yeast extract or other constituent is sufficient to cause both growth and branching" (p. 150). This, of course, assumes that auxin is essential for all branching. If now we turn to the chapter in which the evidence for this assumption is to be found,

that on "root formation," we find (p. 186) that "Sugar solution was found to have no root-forming effect, so that the action is not one of nutrition," and on p. 184 that "Yellow varieties (of *Commelina*) do not root while green ones do, but the difference is not due to nutrition because addition of sugar or peptone does not affect it." Then continuing (p. 186) "It follows from these experiments that root-formation is due to a special substance or hormone; it is not itself a nutrient." This conclusion, which on p. 150 was treated as fact, thus rests on the extraordinarily naïve assumption that in demonstrating the inadequacy of "sugar" and "peptone" to produce rooting, the possibility of nutrition playing a part in the process has been eliminated.

Again, the entire modern quantitative work on "growth hormones" rests on the identification of the four substances isolated from urine (auxin *a*, auxin *b*, and auxin *a* lactone) and from cultures of *Rhizopus* (hetero-auxin) with the effective materials present in *Avena* coleoptiles and other plant tissues. Auxin *a* has not been isolated from such coleoptiles but the molecular weight of the effective material of *Avena* has been determined by diffusion rates to be 376 (Went). The molecular weight of auxin *a* isolated from urine is 328 (Kögl *et al.*). The authors consider that in spite of the 12 per cent discrepancy between these values they are sufficiently close to assume identity. This might have been permissible so long as it appeared that only one substance could produce the result under investigation but in the face of the rapidly accumulating evidence that many substances of widely different structure can produce similar, if not identical, results, it seems to this reviewer that the assumption that "the auxin of coleoptiles is auxin *a*" (p. 114) is *possibly* in accordance with fact, but is certainly based at present on very questionable evidence. It is unfair to the reader to treat such assumptions as fact and to build elaborate theories thereon.

The assumption that the reactions of phanerogamic plants to "hetero-auxin" represent reactions which go on in these plants under natural conditions rests on equally shaky evidence since, so far as this reader can gather from this and other reviews of the subject, *hetero-auxin has never been isolated from such plants* but only from cryptogams. Since such widely different substances as rubidium sulfate and tannic acid will produce similar effects (Avery *et al.*) the identification of hetero-auxin with the naturally occurring "hormone" again appears a bit naïve.

These criticisms must not, however, be taken to disparage the evidential content of the book. The authors have brought together in concise and readable form an enormous mass of observations in a rapidly growing, fascinating and important field. Every plant scientist should find perusal of these pages stimulating and worthwhile. Without proper seasoning with the salt of skepticism, the theories presented are likely to prove indigestible, but if properly seasoned, the content of this volume should prove a nourishing and acceptable morsel.

PHILIP R. WHITE